Chemical Library

OCT 18 1940

SCIENTIFIC EDITION

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

CONTENTS OF THIS ISSUE

	Page
On the Toxicity of Vanillin and Ethyl Vanillin for Rabbits and Rats. *Wilhelm Deichmann and Karl V. Kitzmiller*	425
A Study of Red Squill. *Guilford C. Gross*	428
The Constitution of Celastrol—Part III. *Ole Gisvold*	432
Assay of a Variety of Vitamin B_1 Preparations by the Fluorophotometric Method. *J. W. Cole, W. S. Jones and W. G. Christiansen*	434
A Method for the Determination of Mercury in Complex Ions. *John T. Read and Roger F. Maize*	436
Note on Philippine Turtle Oil. *Pura Villarica and Patrocinio Valenzuela*	437
Studies on Viburnum. IX. *Heber W. Youngken and James C. Munch*	439
Marihuana Investigations. III. *B. B. Robinson and J. R. Matchett*	448
The Clone in Pharmacognosy. *William J. Bonisteel*	453
Drug Extraction. XXIII. The Extraction of Ipomea. *William J. Husa and Thomas J. Macek*	455
Hydrogenated Oil as an Ointment Base. IV. *George W. Fiero and Ted A. Loomis*	458
Poisons and Poisoners. *W. H. Blome and Michael Zajac*	460
Abstracts	429–472
Advertising—Cover pages 2, 3, 4 and pp. I to X.	

Published by the American Pharmaceutical Association. Publication office, 20th and Northampton Sts., Easton, Pa. Editorial office: Scientific Edition, 32 S. Greene St., Baltimore, Md. Practical Pharmacy Edition: 2215 Constitution Ave., Washington, D. C.

Annual Subscription: Journal of the American Pharmaceutical Association, complete $5.00; Canada, $6.00; Other Foreign, $6.50. (*a*) Scientific Edition, $4.00; Canada, $4.35; Other Foreign, $4.50; Single Numbers, $0.35; Canada, $0.40; Other Foreign, $0.50. (*b*) Practical Pharmacy Edition, $1.50; Canada, $1.80; Other Foreign, $2.00; Single Numbers, $0.15; Canada, $0.20; Other Foreign, $0.25.

No claims will be allowed for Journals lost in the mails due to: Failure to notify of change of address (thirty days' notice required); after a lapse of three months; failure to receive wrappers from returned Journals.

Entered as second-class matter January 23, 1917, at the Post Office at Easton, Pa., under the act of March 3, 1879, as 24 times a year: Scientific Edition monthly on the fifth. Practical Pharmacy Edition monthly on the 20th. Acceptance for mailing at a special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized July 10, 1918.

Vol. XXIX, No. 10 OCTOBER, 1940

HOLADIN

THE ENTIRE PANCREAS GLAND SUBSTANCE

In 3 and 5 gr. capsules; and ½ and
1 oz. vials.

GASTRON

AN EXTRACT OF THE ENTIRE GASTRIC GLAND

In 6 oz. and 32 oz. bottles.
Gastron with Iron in 6 oz. bottles.

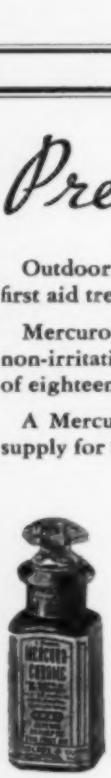
FAIRCHILD BROS. & FOSTER
New York

Prevent Infection

Outdoor activities at this season cause many minor injuries requiring first aid treatment.

Mercurochrome, H. W. & D., supplies your antiseptic needs. It is non-irritating, non-toxic and antiseptic in wounds. It has a background of eighteen years' medical acceptance.

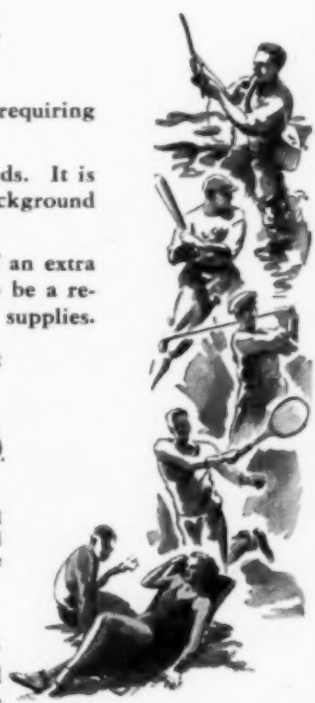
A Mercurochrome display now will suggest the need of an extra supply for first aid kits or camp medicine chests. It will also be a reminder of the seasonal need of other first aid supplies.



An attractive display card and First Aid booklet will be sent to druggists on request.

Mercurochrome. H.W.&D.
(*dibrom-oxymercuri-fluorescein-sodium*)

Every "H. W. & D." product is investigated and proved chemically, bacteriologically and pharmacologically in our laboratories before marketing.



HYNSON, WESTCOTT & DUNNING, INC.
Baltimore, Maryland

Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

A. G. DuMez, Editor, Baltimore, Maryland

VOLUME XXIX | *OCTOBER, 1940* | NUMBER 10 CONSECUTIVE No. 19

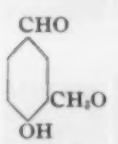
On the Toxicity of Vanillin and Ethyl Vanillin for Rabbits and Rats*

By Wilhelm Deichmann and Karl V. Kitzmiller†

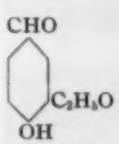
According to Preusse (1) the ingestion of 2 Gm. of vanillin in one dose by a rabbit produced no harmful effects, but repeated doses caused loss of appetite and weight, muscular weakness and death in coma. Dyson (10) set the toxic dose for intravenous administration to white rats at 1.5 Gm. per Kg. A human adult is cited by Kobert (2) as having ingested vanillin in amounts up to one gram without feeling symptoms of discomfort. Despite such evidence that a high dosage is required to produce systemic effects, reports of "vanillin mass poisoning," supposedly due to food flavored by vanilla, found their way into the literature (Kobert (2), Flury and Zangger (3)). The symptoms, which appeared 5–12 hours after the ingestion of food (Rasser (4)), were those of acute gastroenteritis followed, in some instances, by disorders of the central nervous system, and occasionally by death. The mystery of these sudden attacks of poisoning was solved by the discovery of their origin from degradation products of proteins (Vaughan (5)), or from the direct action of microorganisms, primarily those of the paratyphoid group (Wassermann (6), v. Vagdes (7), Curschmann (8), Rasser (4) and Gersbach (9)).

EXPERIMENTAL

The experiments described herein were carried out to determine the acute fatal concentration as well as the effects of the repeated administration of vanillin and ethyl vanillin. The compounds employed for the observations were obtained in a high degree of purity from commercial sources. Chemically, vanillin is 4-hydroxy, 3-methoxy benzaldehyde. Ethyl vanillin, commercially known as Ethavan, is 4-hydroxy, 3-ethoxy benzaldehyde. Its flavoring capacity is approximately 3.5 times that of vanillin.



Vanillin



Ethyl Vanillin

Acute Toxic Dosages of Vanillin and Ethyl Vanillin.—Vanillin and ethyl vanillin were dissolved to the extent of 4% or 5% in milk, by heating slowly to 90°, and cooling to 37°, after which they were administered by stomach tube to albino rabbits or injected subcutaneously into young albino rats. (One rabbit was given a small oral dose in the form

* Presented at the meeting of the American Society for Pharmacology and Experimental Therapeutics, New Orleans meeting, 1940.

† From the Kettering Laboratory of Applied Physiology, University of Cincinnati, Cincinnati, Ohio.

of a 4% solution in olive oil and 2 others received small doses as 0.5% solutions in 10% aqueous glycerol.) The minimal fatal dose of either vanillin or ethyl vanillin, when administered orally to rabbits, was 3.0 Gm. per Kg. of body weight (Table I). Subcutaneous dosages of 2.6 Gm. of vanillin or 2.0 Gm. of ethyl vanillin, per Kg. of body weight, killed 50% of the rats to which they were administered (Table II).

Table I.—Results of Oral Administration of Single Doses of Vanillin and Ethyl Vanillin to Rabbits

Rabbit	Dose, Gm./Kg.	Material Administered	Fate
		Vanillin	
D-338	0.15	0.5% in 10% glycerol	No illness
D-372	0.20	0.5% in 10% glycerol	No illness
N- 24	2.0	5% in milk	Increased respiration, recovery
N- 25	2.5	5% in milk	Increased respiration, collapse, recovery
D-642	3.0	5% in milk	Died in 45 minutes
N- 53	3.5	5% in milk	Died in 45 minutes
D-648	4.0	5% in milk	Died in 110 minutes
		Ethyl Vanillin	
D-337	0.15	4% in olive oil	No illness
D-150	2.5	5% in milk	Increased respiration, recovery
D-721	3.0	5% in milk	Increased respiration, recovery
D-722	3.0	5% in milk	Died in 90 minutes
D-148	4.0	5% in milk	Died in 48 hours
D-149	4.0	5% in milk	Died in 36 hours

Table II.—Results of Subcutaneous Injections of Single Doses of Vanillin and Ethyl Vanillin as 4% Solutions in Milk, into Rats

Number of Animals	Dose, Gm./Kg.	Per Cent Dead	Time till Death
		Vanillin	
10	1.0	..	....
10	1.4	..	....
10	1.8	20	2 hrs. to 4 days
10	2.2	40	2½ hrs. to 4 days
10	2.6	50	2½ hrs. to 4 days
		Ethyl Vanillin	
10	1.0	..	....
10	1.4	..	....
10	1.8	30	7 to 10 hrs.
10	2.0	50	7 to 24 hrs.
10	2.2	80	8 to 30 hrs.
10	2.6	80	3 to 24 hrs.

A fatal dose of vanillin or ethyl vanillin induced an increased rate of respiration followed by muscular weakness, lachrymation, dyspnea, collapse and death in coma. Convulsions were not observed. Sublethal doses produced these signs to a lesser extent, followed by prompt recovery.

Effect of Repeated Administration and Prolonged Feeding of Vanillin and Ethyl Vanillin to Rabbits and Rats.—Vanillin and ethyl vanillin in the form of 4% solutions in milk or olive oil, and 0.5% solutions in 10% aqueous glycerol, were administered to rabbits repeatedly by subcutaneous injection or by means of a stomach tube. No injury or discomfort resulted so long as the dose did not require the administration of toxic amounts of the solvent. Two rabbits died of acute glycerol poisoning evidenced by restlessness, tremor, convulsions and coma.

The effect of prolonged ingestion was studied as follows: To each of 12 rats approximately 300 mg./Kg. of either vanillin or ethyl vanillin, as 4% solutions in olive oil, were administered twice a week for 14 weeks, by means of a blunt hypodermic needle introduced into the esophagus; for 126 days, 16 rats were fed on a normal diet to which vanillin or ethyl vanillin (4% solutions in milk) had been added, each animal consuming about 20 mg./Kg. of vanillin or ethyl vanillin per day; another group of 16 rats was fed as those in Group 2, with the exception that each animal consumed approximately 64 mg./Kg. of vanillin or ethyl vanillin per day, for 70 days. The rats in the first two groups above showed no signs of illness. Their appearance, behavior and gain in weight were normal. Those in the last group appeared normal and lively, but their rate of increase

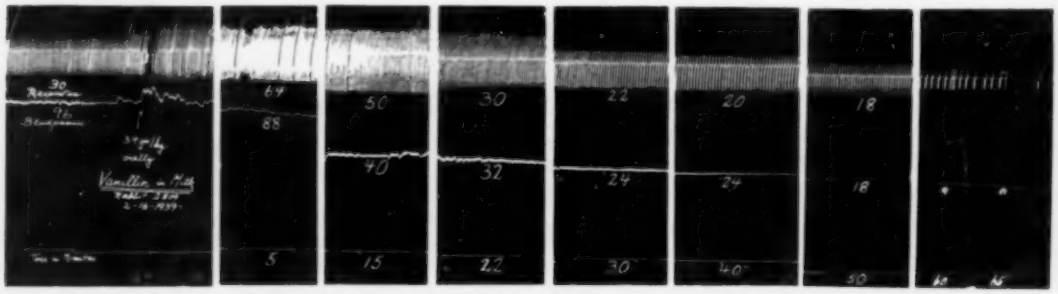


Fig. 1.—The Effect of Vanillin on the Blood Pressure and Respiration of a Rabbit. The Upper Tracing Represents the Respiration, While the Lower Shows the Blood Pressure in Mm. Hg. The Time in Minutes Is Shown on the Bottom Line.

Table III.—Results of Repeated Administrations of Vanillin and Ethyl Vanillin to Rabbits

Rabbit	Number of Doses	Dose, mg./Kg.	Duration of Treatment in Days	Total Amount Administered Gm./Kg.	Solvent Employed	Mode of Application	Fate
				Vanillin			
D- 387	6	153	6	0.9	Olive oil	Subcutaneous	No illness
D- 386	6	165	6	1.0	Olive oil	Subcutaneous	No illness
D- 334	11	83	14	1.16	10% glycerol	Oral	Died
D- 336	51	103	61	6.28	10% glycerol	Oral	Anemia, diarrhea, recovered
D-1299	25	240	56	6.00	Milk	Oral	No illness
D-1297	54	240	126	13.06	Milk	Oral	No illness
D-1298	54	240	126	13.06	Milk	Oral	No illness
				Ethyl Vanillin			
D- 388	6	148	6	0.89	Olive oil	Subcutaneous	No illness
D- 421	6	154	6	0.92	Olive oil	Subcutaneous	No illness
D- 331	13	15	15	0.23	10% glycerol	Oral	Died
D- 377	26	15	31	0.47	10% glycerol	Oral	No illness
D- 332	15	32	17	0.54	10% glycerol	Oral	No illness
D- 378	26	41	31	1.27	10% glycerol	Oral	No illness
D- 333	43	49	49	2.40	10% glycerol	Oral	Anemia, diarrhea, no gain in wt.
D-1302	25	240	56	6.00	Milk	Oral	No illness
D-1300	54	240	126	13.06	Milk	Oral	No illness
D-1301	54	240	126	13.06	Milk	Oral	No illness

Table IV.—Effect of Repeated Oral Administration and Prolonged Feeding of Vanillin and Ethyl Vanillin on the Increase in Weight of Rats

	Vanillin			Ethyl Vanillin		
Time	(6 Rats) 300 mg./Kg. Orally in Olive Oil Twice a Week for 14 Weeks	(8 Rats) 20 mg./Kg. Daily for 126 Days with Diet	(8 Rats) 64 mg./Kg. Daily for 70 Days with Diet	(6 Rats) 300 mg./Kg. Orally in Olive Oil Twice a Week for 14 Weeks	(8 Rats) 20 mg./Kg. Daily for 126 Days with Diet	(8 Rats) 64 mg./Kg. Daily for 70 Days with Diet
Control weight	168	165	129	178	158	133
Wt. after 2 weeks	188	208	141	196	196	155
Wt. after 6 weeks	253	261	166	259	253	167
Wt. after 10 weeks	296	284	173[a]	297	281	190[a]
Wt. after 14 weeks	334	305	182[a]	320	310	202[a]
Wt. after 18 weeks	..	323	193[a]	..	334	212[a]

[a] Animals on normal diet.

in weight was retarded. At the end of the 70-day period 8 of these animals were sacrificed for histopathological study, and the remaining 8 were changed to a normal diet. The rate of weight increase of the latter did not accelerate, and it would appear therefore that they had suffered some systemic damage from the daily dosage of approximately 64 mg./Kg. of one or other of these compounds.

The Effect of Vanillin and Ethyl Vanillin on the Blood Pressure and Respiration.—Rabbits anesthetized by an intraperitoneal injection of 0.2 Gm. per Kg. sodium barbital were employed to obtain tracings of the carotid blood pressure and the respiration. The compounds were administered as a 5% solution in milk (prepared as above) by means of a stomach tube. A lethal dose of vanillin produced a sudden (within the first 10 minutes) drop of blood pressure from an average of 100 to 40 mm. Hg. The rate of respiration usually doubled during the first 10 minutes, then fell just as quickly to the normal level and remained there until shortly before the animal died. The blood pressure continued to fall, slowly but persistently, over a period of from 40 to 110 minutes, finally causing death. Sublethal doses also produced sudden depression of the blood pressure and stimulation of the respiration.

Lethal doses of ethyl vanillin produced essentially the same results as vanillin except that the onset of change in the respiration and blood pressure was much more gradual. A barely sublethal dose had no effect on the respiration over a period of 5 hours, and there was no well-defined effect on the blood pressure.

Gross Pathologic Changes Induced by Vanillin and Ethyl Vanillin.—The pathologic picture in acutely poisoned animals was that of pronounced congestion of the liver and a well-marked dilatation of the abdominal and mesenteric vessels, with little evidence of changes in other tissues, except for the edema at the site of subcutaneous injections and an intense irritation of the gastro-enteric tract in animals given fatal oral doses. The rats given repeated oral doses or fed vanillin and ethyl vanillin with the diet showed no gross evidence of injurious effects.

Microscopic Pathologic Changes.—The histopathologic tissue changes were essentially of the same type in both rabbits and rats regardless of the mode of administration of vanillin and ethyl vanillin. They were those of severe toxemia, involving chiefly the

myocardium, lung, liver and kidney, upon which were superimposed the effects of circulatory failure incident to myocardial damage, and, in many instances, secondary lobular pneumonia.

Lethal and slightly sublethal doses produced cloudy swelling, loss of cross striations and sometimes coagulation necrosis of the fibers of the myocardium, with swelling and pyknosis of the nuclei, edema and slight leucocytic infiltration of interstitial tissue, and edema of the vascular endothelium.

Similar effects, varying only in degree and in accord with the architecture of the organ, were seen in the lung, liver and kidney. Acute passive congestion was the most striking picture in the spleen, central degeneration (often of the fatty type) or necrosis was the most prominent lesion in the liver, while generalized cloudy swelling and degeneration of the tubal epithelium characterized the kidney changes.

Such damage as occurred in the stomach and small intestine resulted in edema and superficial desquamation of the mucosa, with some evidences of congestion and acute exudative reaction in the submucosa.

The animals to which repeated doses of vanillin and ethyl vanillin were administered or fed in dosages above 20 mg. per Kg. of body weight showed definite toxic changes of the foregoing type in their tissues. The rats given daily doses of 20 mg./Kg. for $4^1/_2$ months failed to develop pathologic processes of greater severity or frequency than those commonly found in control animals as the result of spontaneous disease or other unrecognized environmental influences.

Discussion.—The daily ingestion of 20 mg./Kg. of vanillin and ethyl vanillin by rats over a period of 126 days was found to be harmless. Higher doses produced histopathological changes of varying degrees of severity in the myocardium, kidney, liver, lungs, spleen and stomach of rats and rabbits.

It is evident that these compounds are capable of causing fatal poisoning but their relative insolubility in cold water or in cold milk and the bitter taste of concentrated extracts make the possibility of such an event very remote. Concentrations up to 0.5 Gm. of vanillin or 0.15 Gm. of ethyl vanillin per quart are added to foods, and in view of current dietary usage or even extreme individual predilections for flavored foods, injurious effects from prolonged consumption of these compounds are not to be anticipated.

Most of the vanillin is oxidized in the animal body to vanillic acid, which is excreted with the urine as conjugated sulfate. Traces of vanillin and vanillic acid are excreted as such (Preusse (1)).

CONCLUSION

When ingested in high concentration vanillin and ethyl vanillin induce an acute toxemia with death by circulatory failure. Experimental observations on rabbits and rats indicate that the quantities which are employed in foods as flavoring materials are harmless.

REFERENCES

(1) Preusse, C., *Z. physiol. Chem.*, 4 (1880) 209.
(2) Kobert, R., "Lehrbuch der Intoxikationen II," Stuttgart (1906).
(3) Flury, F., and Zangger, H., "Lehrbuch der Toxikologie," Berlin (1928).
(4) Rasser, E. O., *Z. f. Krankenanstalten, Leipzig*, 11 (1915), 266.
(5) Vaughan, cited by Kobert, "Lehrbuch der Intoxikationen II," Stuttgart (1906).
(6) Wassermann, cited by Flury, F., and Zangger, H., "Lehrbuch der Toxikologie," Berlin (1928).
(7) v. Vagdes, cited by Flury, F., and Zangger, H., "Lehrbuch der Toxikologie," Berlin (1928).
(8) Curschmann, cited by Flury, F., and Zangger, H., "Lehrbuch der Toxikologie," Berlin (1928).
(9) Gersbach, A., *Klin. Wochschr.*, 3 (1924), 1278.
(10) Dyson, G. M., *Perfumer. Essent. Oil Record*, 22 (1931), 4.

A Study of Red Squill*

By Guilford C. Gross†

The use of red squill as a rat poison has become very popular in recent years. A great share of this popularity is due, no doubt, to the fact that while red squill is extremely toxic to rodents, it is relatively non-toxic to other wild and domestic animals and human beings. The other rodent poisons in general use to-day (arsenic, barium carbonate, phosphorus and strychnine) have the disadvantage of being toxic to all forms of animal life.

The development of red squill as a specific rat poison has been greatly retarded until recent years because of the difficulty which was experienced in obtaining a uniformly toxic preparation. However, recently there has been considerable investigation conducted with reference to the toxic nature of the rat-killing principle in red squill and as a result this difficulty has been largely overcome.

---

* Kilmer Paper, 1939.
† Instructor in Pharmacy, South Dakota State College.

It is interesting to note that while red squill is of the same species of *Urginea* as white squill, the latter is not effective as a rat poison. It is thus apparent that red squill contains an additional principle which is toxic to rats. In this respect, Winton (1) states that the cardiac glucoside and rat-killing principle in red squill are distinct substances. Both of these substances

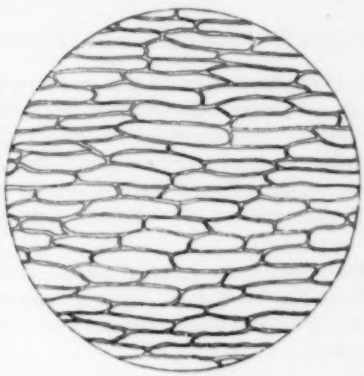


Fig. 1.—Red Squill Bulb Scale. Surface Section.

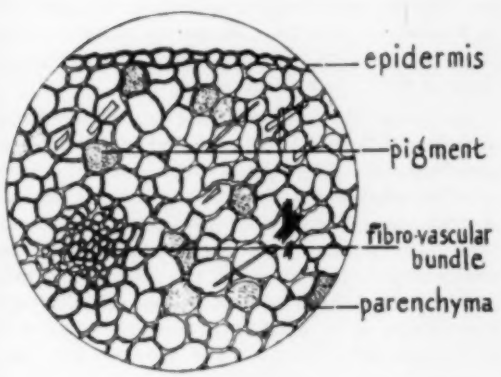


Fig. 2.—Red Squill Bulb Scale. Cross Section.

are found in red squill while white squill does not contain significant amounts of the rat-killing principle.

Both squills have essentially the same medicinal action, but differ in regard to rat-killing properties.

EXPERIMENTAL

HISTOLOGICAL STUDY

A microscopical study of red squill reveals the same structures found in the white variety, but, in addition, red pigment cells are present in the red variety. G. Keenan (2) of the Food, Drug and Insecticide Administration has made a microscopical comparison of red and white squill powders. He states that the greatest diagnostic difference is the presence of pigment cells in the red squill scales and their absence in the white.

Sections of the scales of the fresh bulb of red squill were cut and studied microscopically. The following structures were noted:

Epidermis: Epidermis of thin-walled cells, stomata present.

Mesophyll: Mesophyll of thin-walled cells, irregular in shape, parenchymatous in nature.

Fibrovascular Bundles: Fibrovascular bundles scattered throughout mesophyll. Spiral tracheæ were observed.

Pigment Cells: Cells containing a red pigment were found scattered throughout the mesophyll.

Crystals: Raphides of calcium oxalate were numerous. The crystals varied greatly in length.

Drawings of the bulb scales of red squill, Figs. 1, 2, 3 and 4, show these structures.

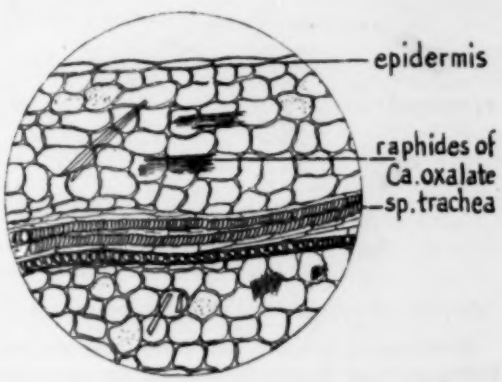


Fig. 3.—Red Squill Bulb Scale. Longitudinal Section.

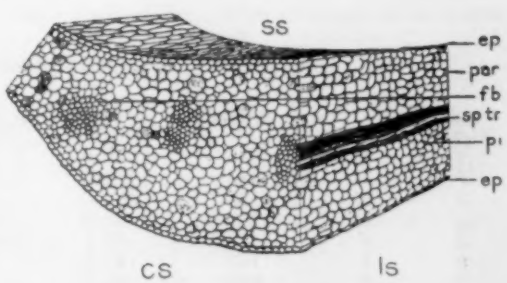


Fig. 4.—Red Squill Bulb Scale.

ss = surface section, *cs* = cross section, *ls* = longitudinal section, *ep* = epidermis, *pi* = pigment, *par* = parenchyma, *sp* = spiral trachea, *fb* = fibro-vascular bundle.

A microscopical examination for comparison of red squill and white squill powders was made. Other than the presence of red pigment cells in red

squill powder, no important differences in the appearance of the two powders could be detected as is shown in Figs. 5 and 6.

Fig. 5.—Red Squill Powder.

Fig. 6.—White Squill Powder.

EXTRACTION STUDIES AND TOXICITY EXPERIMENTS

Experimental evidence has shown that the rat-killing principle is present in significant quantities only in the red variety of squill. The isolation of this principle was conducted according to the method suggested by LeBlanc (3) and which, in his work, gave a product approximately 100 times as potent as the original powder.

Before extraction studies were begun, a commercial sample of red squill was fed to 8 rats to determine its approximate toxicity. The product used contained 10% of red squill powder in what appeared to be a base of corn meal. It was fed to both male and female rats. In all cases, the animals were starved for 24 hours prior to feedings. The powder was further diluted with corn meal at the time of administration. Results of the experiment are shown in Table I.

From the above table, it may be noted that, while only in one instance did the product prove fatal to male rats when fed at the rate of 100 mg. of red squill powder per Kg., the product did appear to be quite toxic since male rats receiving doses of 50 mg./Kg. became very sick. Female rats appeared to be more susceptible. All of those receiving doses of 100 mg. of red squill powder per Kg. died and one receiving a dose of 50 mg./Kg. also succumbed.

EXTRACTION OF THE RAT-KILLING PRINCIPLE

LeBlanc (3) has suggested the use of 80% ethyl alcohol in the extraction of the rat-killing principle from powdered red squill. The extract thus obtained is further purified using animal charcoal and petroleum ether. The procedure used here was essentially the same. The following steps were involved:

(*a*) *Extraction of the Active Principle Using 80% Ethyl Alcohol as the Solvent.*—The extract thus obtained was dried at a temperature not exceeding 80° C. and powdered.

(*b*) *Purification of the Extract Using Animal Charcoal.*—An aqueous solution of the extract obtained above was shaken with animal charcoal. The animal charcoal adsorbed the rat-killing principle, and it is removed from the charcoal by agitation with 80% ethyl alcohol. The product thus obtained is dried at 80° C and powdered.

(*c*) *Further Purification of the Potent Extract Using Petroleum Ether.*—The extract obtained above was dissolved in 80% alcohol and to this an equal volume of ethyl ether was added and the mixture shaken. A small amount of material was thrown out of solution. The mixture was filtered and the filtrate evaporated to dryness at 80° C. The extract thus obtained was shaken with petroleum ether for several minutes to remove a small amount of a fixed oil present as an impurity. The mixture was then filtered and the potent principle allowed to dry spontaneously. The product thus obtained represents a concentrated form of the rat-killing principle.

Table I.—Results of Toxicity Tests

Rat Number	Sex	Weight, Gm.	Amount Powder mg./Kg.	Dilution	Amount Squill, mg./Kg.	Total mg. Squill Fed per Rat	Results
1	M	325	325.0	1:10	100	32.5	Killed
2	M	285	142.5	1:10	50	14.2	Sick, recovered
3	M	185	185.0	1:10	100	18.5	Sick, recovered
4	M	132	66.0	1:10	50	6.6	Sick, recovered
5	F	170	85.0	1:10	50	8.5	Killed
6	F	143	143.0	1:10	100	14.3	Killed
7	F	285	285.0	1:10	100	28.5	Killed
8	F	142	71.0	1:10	50	7.1	Sick, recovered

POTENCY OF THE VARIOUS EXTRACTS

The extract obtained under (*b*) was fed in varying quantities to previously starved rats. Prior to feed-

ing, the product was mixed with powdered oatmeal and moistened slightly. The results are shown in Table II.

Table II.—Potency of Extracts

Rat Number	Sex	Weight, Gm.	Amount Extract, mg./Kg.	Total mg. Extract Fed per Rat	Results
9	M	287	100	28.7	Died
10	M	190	25	4.7	Died
11	M	244	15	3.7	Died
12[a]	M	352	10	3.5	Lived
13	M	329	5	1.6	Died

[a] Rat No. 12 ate less than one-half of the bait.

To determine whether or not the red squill extract obtained above is toxic to rats when fed at less than 5 mg./Kg., four rats were weighed, starved and fed graduated doses of the extract. A 1–5 dilution of extract was used in feeding and this was further diluted with oatmeal at the time of administration. The diluent used in the first case was lactose. Results of the experiment are shown in Table III.

Table III.—Potency of Lactose Dilutions

Rat Number	Sex	Weight Gm.	Amount of Dilution Fed mg./Kg.	Dilution	Amount Extract mg./Kg.	Total mg. Extract per Rat	Results
17	F	317	25	1:5	5	1.58	Died
18	M	272	20	1:5	4	1.08	Lived
19	M	243	15	1:5	3	0.73	Lived
20	M	232	10	1:5	2	0.46	Lived

From the foregoing experiments it may be estimated that the minimum lethal dose of the extract for male rats is somewhere in the vicinity of 5 mg./Kg.

In order to determine the toxicity of the purified extract obtained under (*c*), 4 male rats were weighed, starved and fed graduated doses of the extract. The extract was diluted 1–10 with lactose. At the time of administration, it was further diluted with oatmeal. Results of the feeding are shown in Table IV.

Table IV.—Potency of Dilutions of Purified Extract

Rat Number	Sex	Weight, Gm.	Amount of Dilution Fed, mg./Kg.	Dilution	Amount Extract, mg./Kg.	Total Mg. Extract per Rat	Results
21	M	280	50	1:10	5	1.40	Died
22	M	224	40	1:10	4	0.89	Died
23	M	241	30	1:10	3	0.72	Lived
24	M	225	20	1:10	2	0.45	Lived

The results shown in Table IV indicate that the minimum lethal dose for male rats lies between 3 and 5 mg./Kg. However, the small number of experiments does not warrant arriving at any definite conclusion. The toxicity to female rats of the extract obtained under (*c*) was not determined but it would apparently be somewhat below the above figures.

PHYSIOLOGICAL ACTION OF THE RAT-KILLING PRINCIPLE ON RATS

The physiological action of the extract (*b*) was observed on the rats fed in Table II. Within 2 hours after feeding the red squill extract, rats Nos. 9, 10 and 11 (Table II) exhibited symptoms of poisoning. They became lethargic, with rat No. 9 exhibiting marked inflammation of the area of the nose and mouth due to the irritant action of the squill. This inflammation was not so noticeable on the 10th and 11th rats. The rats did not readily respond to stimuli although, when the cages were tapped, they jerked slightly. They sneezed considerably. Within 4 hours, all rats except No. 12 were very sick. They were slightly more excitable but remained quiet when not disturbed. Respiration increased in rate and appeared to be labored. The characteristic gyrating symptoms were observed 6 hours after administration in rats Nos. 9, 10 and 11. Gyrations occurred at short intervals. The application of a slight stimulus, such as tapping the cage, caused a resting rat to promptly resume its gyrating movements. At times the rats went through the motions of vomiting but were unable to do so. Rat No. 13 showed symptoms of poisoning but these were not as marked as in the first three cases. Rat No. 12 appeared to be in fairly healthy condition.

When it was definitely established that rats Nos. 9, 10 and 11 would not live much longer, the following experiment was carried out, the purpose being to determine, if possible, whether or not the rat-killing principle is absorbed. One cc. of blood was taken from the heart of rat No. 9 and injected intraperitoneally into a healthy rat (rat No. 14). Likewise, $2^1/_2$ cc. of blood were drawn from rat No. 10 and $1^1/_2$ cc. from rat No. 11 and injected into 2 healthy rats (rats Nos. 15 and 16, respectively). All of the injected rats lived, indicating that there is no absorption of the rat-killing principle.

PHYSIOLOGICAL ACTION OF RAT-KILLING PRINCIPLE ON THE DOG

A dog weighing 15.9 Kg. was fed red squill extract (*b*) at the rate of 48.4 mg./Kg. It was mixed with ground dog food and fed late one afternoon. The dog took several bites of the treated food and then refused to eat the remainder. No symptoms of poisoning other than salivation were noted immediately following administration. The next morning, how-

ever, when the dog was removed from the cage, it was observed that he staggered slightly as he walked. At this time the same dose of the extract previously given was administered by means of a stomach tube. The first symptoms of poisoning noted were intense salivation and vomiting. These appeared shortly after the administration of the poison. The heartbeat appeared to become somewhat slower and irregular, but it was difficult to make an accurate count. The dog attempted to vomit on several occasions but was unable to do so. At 27 minutes after administration, the animal experienced severe convulsions and died within several minutes. Postmortem examination revealed the heart stopped in systole. It is believed that heart action and respiration ceased almost simultaneously.

SUMMARY

1. The structure of the inner scale of the bulb of red squill was studied microscopically.

2. Powdered red squill and powdered white squill were compared under the microscope. The presence of fragments of red pigmented tissue in the red squill powder served as the only diagnostic difference between the two powders.

3. The active rat-killing principle was extracted using 80% ethyl alcohol as the solvent. This, on subsequent purification with animal charcoal, ether and petroleum ether, yielded a product which caused death in a male rat when fed at the rate of 4 mg./Kg.

4. The physiological action of the rat-killing principle on rats and dogs was observed.

REFERENCES

(1) Winton, F. R., "The Rat Poisoning Substance in Red Squill," *J. Pharmacol.*, 31 (1928), 123; *Pharm. J.*, 120 (1928), 73; 122 (1928), 236. "White Squill and Its Preparations Useless as Rat Poisons," *J. Pharmacol.*, 31 (1928), 137; *Pharm. J.*, 120 (1928), 55.

(2) Keenan, G. L., U. S. Dept. Agr. Tech. Bull. (1929), page 134.

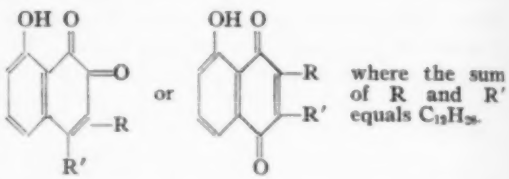
(3) LeBlanc, F. J., Thesis, Purdue University, (1938).

Wilhelm Ostwald (1853–1932) was awarded the Nobel Prize for Chemistry in 1909 in recognition of his work on catalysis and for his investigations in the fields of chemical equilibria and reaction velocities.

The Constitution of Celastrol—Part III

*By Ole Gisvold**

In previous publications (1) it was shown that celastrol, a pigment found in the outer bark of the root of *Celastrus Scandens*, has the formula $C_{29}H_{30}O_3$. One oxygen was reported present as a hydroxyl group and the remaining two oxygens as possibly being in the form of an ortho quinone. Subsequent investigations on the constitution of this pigment indicate that it might possibly be an alkyl substituted 5-hydroxy-1, 4-naphthoquinone with the following tentative formulas



where the sum of R and R' equals $C_{19}H_{28}$.

Permanganate oxidation of celastrol yielded a small quantity of a crystalline material that was best characterized as 3-hydroxy phthalic acid. Attempts to obtain more of this compound or its methoxy derivative by oxidation with chromic acid did not prove successful. However, all crude oxidation materials gave a positive fluorescein test, with resorcinol when tested for phthalates.

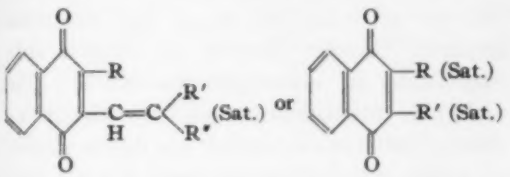
Celastrol does not form a water-soluble bisulfite addition product and only a minute quantity of a phenazine. It can be reduced with sulfurous acid and reoxidized by atmospheric oxygen after the removal of sulfur dioxide.

Celastrol is orange and, according to Hooker, (2) 2 hydroxy naphthoquinones which have a side chain in position, 3 are yellow if a double bond is present in the β,γ-position and red to orange if the double bond is in the α,β-position. Ultimate analysis of celastrol indicates that it does not have a double bond in the side chain. This is also strengthened by the fact that celastrol when reduced with Raney nickel at 190° in alcohol is converted to its original color upon

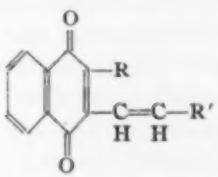
* From the Department of Pharmaceutical Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota.

spontaneous oxidation in air. If a double bond were present in the α,β-position which was contributing to the orange-red color of celastrol, it would have to be inert to the drastic hydrogenation to which it has been subjected.

Celastrol or methyl celastrol fail to give a violet color test with 1 per cent sodium methoxide. Compounds with the following constitution give no color or at best a weak blue (3).



α-Naphthoquinones with the following constitution give an intense purple or blue color test with 1 per cent sodium methoxide (4).



According to Fieser (5), those naphthoquinones examined which have no activating double bond or phenyl group attached to the α-carbon atom of the side chain do not undergo an abnormal acylation. Although celastrol apparently conforms to the above requirements, nevertheless it forms a yellow acetate which is indicative of the abnormal acylations that is characteristic of the naphthoquinones investigated for this property.

When tested for its antihemorrhagic activity, celastrol proved to be entirely negative in effect in doses as high as 100 mg. per Kg. Methyl celastrol was inert in all doses below 25 mg. per Kg., although a slight effect was evident at the 25-mg. dose.[1]

EXPERIMENTAL

Oxidation with Potassium Permanganate.—Five grams of celastrol were oxidized with aqueous alkaline permanganate according to R. Kuhn and A. Deutsch (6). All attempts to obtain a crystalline product by crystallization methods failed. The following technique overcame this difficulty. Purified sand was impregnated with the oxidized material and then extracted with Skelly-solve *B*. The Skelly-solve *B* soluble fraction was crystallized from dry ether. The crystalline material obtained in this way had an aromatic odor which was characteristic of the material still remaining upon the sand. The material when crystallized from water was odorless, colorless and, when dried, melted at 245° C. It was difficult to obtain a sharp melting point. This material could be readily sublimed and the crystalline sublimate melted at 242–244° C. It gave a positive fluorescein reaction.

Analysis:

$C_8H_6O_3$	Calc.	C	52.6	H	3.3
	Found	C	52.20	H	3.14
			52.22		2.90

Molecular weight, rast 190

No color was obtained when it was tested with ferric chloride. 3-Hydroxyphthalic acid is reported as melting at 161° to 163° C. and its anhydride at 198° to 199° C. (7). It is reported as melting at 244° C. in the "Handbook of Chemistry and Physics."

Oxidation of Celastrol with Chromic Acid in Glacial Acetic Acid.—In an attempt to prepare more phthalic acid or a phthalic acid derivative, celastrol was oxidized with chromic acid in boiling acetic acid (8). One gram of celastrol was refluxed with 3 Gm. of chromic acid in glacial acetic acid for about one and one-half hours. No crystalline material could be obtained from the sodium bicarbonate soluble fraction of the oxidized material. However, it gave a positive fluorescein reaction. Successive reoxidations of the oxidized material by increasing the amounts of chromic acid did not lead to the successful isolation of a crystalline substance. In fact, such treatment resulted in the complete loss of any ether-soluble material.

Oxidation of Methyl Celastrol with Chromic Acid in Glacial Acetic Acid.—The same procedure described above was used in the oxidation of methyl celastrol. No crystalline oxidation substance could be obtained from the oxidation material. It gave a positive fluorescein reaction. Successive reoxidations of the oxidized material by increasing the amounts of chromic acid did not lead to the successful isolation of a crystalline substance. In fact, such treatment resulted in the complete loss of any ether-soluble material, but this destruction proceeded at a slower rate than with celastrol.

Reductive Acetylation.—Methyl celastrol was mixed with zinc dust, acetic acid and acetic anhydride. The mixture was warmed on the steam-bath for two hours. The reaction mixture remained colorless as long as the evolution of hydrogen took place. The reaction mixture was filtered and the slightly yellow filtrate intensified in color upon exposure to air and removal of the solvent.

[1] Marvin R. Thompson personal communication through the courtesy of the Warner Institute for Therapeutic Research.

This derivative separated from anhydrous ether in the form of a crystalline powder which melted at 210° C.

Analysis: CH_3CO

$C_{27}H_{38}O_3$ Calc. for 2 acetyl groups 19.50 per cent
Found 19.68, 22.67, av. 21.17 per cent

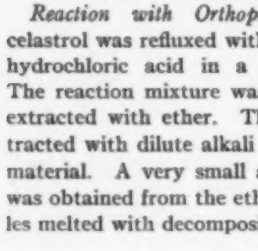
Reaction with Bisulfite.—Celastrol was found to be insoluble in a saturated solution of sodium bisulfite and it cannot be extracted from an ether solution with this reagent.

Reaction with Sulfurous Acid.—An alcohol solution of either methyl celastrol or celastrol became colorless upon the addition of sulfurous acid. The solutions became colored again upon removal of the sulfur dioxide and aeration of the mixture.

Reaction with Orthophenylenediamine.—Methyl celastrol was refluxed with *o*-phenylenediamine and hydrochloric acid in a hydro-alcoholic solution. The reaction mixture was diluted with water and extracted with ether. The ether solution was extracted with dilute alkali to remove any unreacted material. A very small amount of yellow needles was obtained from the ether solution. These needles melted with decomposition at 275° to 285° C.

SUMMARY

The evidence presented in Papers I and II together with subsequent investigations upon the constitution of celastrol indicates that it has the formula $C_{29}H_{38}O_3$. It is either a mono- or dialkyl substituted β- or α-naphthcquinone of one of the following tentative formulas:



where the sum of R and R′ equals $C_{13}H_{18}$.

REFERENCES

(1) O. Giswold, JOUR. A. PH. A., 28 (1939), 440; 29 (1940), 12.

(2) Hooker, S. C., *J. Am. Chem. Soc.*, 58 (1936), 1163.

(3) Fieser, L. F., *et al.*, *Ibid.*, 61 (1939), 3216.

(4) Fieser, L. F., *Ibid.*, 61 (1939), 2213; Hooker, S. C., *Ibid.*, 58 (1936), 1163.

(5) Fieser, L. F., *Ibid.*, 48 (1926), 3201; *Ibid.*, 61 (1939), 2213.

(6) *Ber.*, 66 (1933), 883.

(7) Pratt, D. S., and Perkins, G. A., *J. Am. Chem. Soc.*, 40 (1918), 228.

(8) MacCorquodale, *et al.*, *J. Biol. Chem.*, 131 (1939), 362.

A postage stamp in honor of the famous botanist, Karl von Linné has been issued by Sweden.

Assay of a Variety of Vitamin B_1 Preparations by the Fluorophotometric Method

*By J. W. Cole, W. S. Jones and W. G. Christiansen**

Until recent years one has had to rely on biological methods for the estimation or determination of vitamin B_1. The disadvantages of such a situation are obvious; the cost of biological assays and the time required became factors of considerable magnitude in investigational work. The isolation, identification and synthesis of thiamin have made possible the development of rapid physiochemical methods for the estimation of vitamin B_1. While the status of these chemical methods is at present not such that they can replace the biological assay as a basic standard for establishing the B_1 potency of a product and while it is questionable as to whether that ever should be so, because the products being dealt with are intended to produce certain biological responses or effects when fed to humans (or animals), the very great usefulness of a rapid, reliable chemical method for investigational work, process control, preliminary testing so as to reduce to a minimum the amount of biological testing, stability under storage conditions, etc., is readily appreciated.

Thiamin, in both the free and phosphorylated form, is oxidized, respectively, to the free and phosphorylated thiochrome by potassium ferricyanide in alkaline solution. The free thiochrome is readily extracted by isobutanol and gives a violet-blue fluorescence in ultraviolet light. The phosphorylated thiochrome is not extracted by isobutanol. However, when the thiamin in the material to be tested exists wholly or partially in the phosphorylated form, one can by enzymatic hydrolysis, as the first step in the assay, convert the phosphorylated form into free thiamin and thus make possible the recovery of all the thiamin in the form of free thiochrome for estimation

* Control and Development Divisions, Chemical and Pharmaceutical Laboratories. E. R. Squibb & Sons, Brooklyn, N. Y.

fluorometrically. This serves as a means of learning the total thiamin content; a similar assay without enzymatic hydrolysis reveals the amount present in the free form, and the difference obviously represents thiamin present in the phosphorylated form. It is possible, therefore, to determine the character of the thiamin in the material as well as the total amount present.

fluorophotometer (Model A) manufactured by Pfaltz & Bauer, Inc., operates on the principle that an exciting beam from the light source passes through the isobutanol solution of thiochrome in a cuvette causing it to fluoresce. The fluorescent light strikes a photocell, which transforms the light into electric energy; this photoelectric current is then registered on the galvanometer.

Table I.—Thiamin Chloride and Preparations Containing It as the Only Vitamin Component

Form of the Thiamin Chloride	No. of Samples Tested	Fluorophotometric Assay			Calculated Potency*
		Minimum	Maximum	Average	
Powder	12	933,000	1,041,000	999,000	1,000,000 γ/Gm.
Tablet	6	1,017	1,170	1,062	1,000 γ/tab.
Tablet	1	2,880	3,300	3,053	3,000 γ/tab.
Tablet	6	4,840	5,300	5,010	5,000 γ/tab.
Tablet	1	....	....	9,890	10,000 γ/tab.
Solution	5	10,000	11,800	10,200	10,000 γ/cc.
Solution	2	49,000	51,800	50,400	50,000 γ/cc.
Elixir	36	2,720	3,800	3,250	3,060 γ/fl. oz.
Mineral oil emulsion	2	1,207	1,224	1,215	1,150 γ/fl. oz.

* Calculated on a basis of the amount of thiamin chloride in the sample tested.

By applying these principles Hennessy and Cerecedo[1] have developed a simple method or procedure for the quantitative estimation of vitamin B_1 fluorophotometrically. One of the important aspects of this method is that it provides a means for the assaying of complex mixtures. With thiamin alone, free from the materials with which it is associated in natural products or the materials with which it is mixed in the various multi-vitamin preparations, the estimation is reasonably straightforward, but when these other materials are present, interference is so great that the results are of little value. By contacting the solution containing the thiamin with zeolite, the "base exchange" characteristics of the latter result in the replacement by thiamin of one of the bases in the zeolite, remaining in the latter and being available for elution by a suitable liquid. This eluate contains the thiamin without the interfering substances and the assay can be made without difficulty. Hennessy and Cerecedo have published the details of the procedure and have reported on it at various scientific meetings during the last several years; it is, therefore, unnecessary to repeat them here. The instrument used, a

[1] *J. Am. Chem. Soc.*, 61, (1939) 179; A. C. S. Abstracts of Meeting, Baltimore, April 3 to 7, 1939, Division of Biological Chemistry.

For hydrolysis of any phosphorylated thiamin an enzyme preparation from beef kidney was formerly used by Hennessy; takadiastase is now used and is found satisfactory—the hydrolysis is accomplished in one and one-half hours at 40° C.

EXPERIMENTAL

For more than a year, this laboratory has been routinely applying the fluorophotometric method of Hennessy to a wide variety of raw materials, mix-

Table II.—Natural Materials Containing the B Complex

Material Tested	Vitamin B_1 in Gamma per Gm.	
	Fluorophotometric Assay	Bioassay
Yeast powder	181	156
Yeast powder	177	165
Yeast powder	150	156
Yeast powder	181	180
Wheat germ	18.6	15
Malted wheat germ extract		
Powder	23.8	21.0
Powder	20.4	24.9
Syrup	11.4	15.0
Concentrated cereal extract	162	186
Concentrated cereal extract	174	180
Concentrated cereal extract	151	150
Concentrated cereal extract	156	150
Powder containing B complex	633	660
Powder containing B complex	624	660
Powder containing B complex	602	660

Table III.—Preparations Made Using Bioassayed Natural Materials

Preparation Tested	No. of Samples Tested	Fluorophotometric Assay Minimum	Maximum	Average	Calculated Potency[a]
A. Uni-vitamin products					
Yeast tablets	8	52.5	68.4	60.6	60 γ/tab.
Yeast tablets	33	47.4	79.2	58.5	63 γ/tab.
Malted wheat germ extract cpd	17	6.12	9.3	6.96	6.4 γ/Gm.
B. Multi-vitamin products					
Yeast-fish liver oil tablets	6	132	255	181.5	172.5 γ/tab.
Paste in capsules, Type I	18	93	119	105	114 γ/cap.
Paste in capsules, Type II	9	702	864	774	798 γ/cap.
Paste in capsules, Type III	4	798	933	879	900 γ/cap.
Paste in capsules, Type IV	10	95	130	111	104 γ/cap.

[a] Calculated from the bioassay on the ingredients.

tures and finished products. For convenience of presentation the materials tested have been divided into three types and the data on each are shown in a separate table. (See Tables I, II and III.) These tabulated data require little comment. As would be expected there are variations in the results obtained by the fluorophotometric assay and there are differences between them and those obtained by bioassay or by calculation from potency data on the B_1 components of the products tested. However, taken as a whole findings are good, and it does appear that this fluorophotometric method is of practical value in testing for vitamin B_1.

One of the most important phases of this kind of work is the preparation of the sample for test. That is, of course, an easy matter when one is dealing with thiamin chloride itself or the simple forms of it such as solutions and tablets but becomes a very difficult matter when one reaches the complex multivitamin preparations such as the various pastes in capsules—obviously there are products representing practically the entire range from those easy to handle to the difficult ones. Inasmuch as each product or mixture is a problem in itself and the preparation for analysis has to be adjusted in accordance with the character and composition of the material to be tested and inasmuch as it is not the purpose of this paper to take up that phase of the work details on how to go about assaying, each type of sample will not be given. The point is that even though the complexity of the mixture does become high the assay can be run and results of the proper order are obtained.

Table III, Section *B*, relates to multi-vitamin preparations in which the fluorophotometric data were compared with figures calculated from the bioassay data as components of the mixtures instead of with the findings in bioassays on the mixture itself. For this reason and because of the difficulties associated with the assaying of these complex mixtures (see above), it is worth while to include a comparison of the fluorophotometric assay with the bioassay of the finished complex mixture. In a multivitamin paste in capsules the fluorophotometric assay showed 108 gamma of B_1 per capsule as compared with 99 by bioassay. In a syrupy emulsion of fish liver oil and other fats containing the *B* complex, carbohydrates, proteins, minerals and flavoring materials, the two samples gave the following results:

	Fluorophotometric	Biological
A	423 γ/fl. oz.	450 γ/fl. oz.
B	540	465

Note that when the proper method of handling mixtures like these two have been satisfactorily worked out and skill in the technique has been developed close agreement with bioassay can be had.

A Method for the Determination of Mercury in Complex Ions*

By John T. Read and Roger F. Maize†

The determination of mercury in complex anions such as that occurring in potassium mercuric iodide presents a problem for which ordinary methods of analysis and available literature fail to give a satisfactory solution, the definite problem at hand being the estimation of mercury in germicidal tablets containing mercuric iodide, potassium iodide, sodium bicarbonate and an organic dye. The major difficulty consists of splitting the complex ion containing the mercury and converting the latter into a form in which it can be readily estimated.

METHOD DEVELOPED

The details of the method as finally developed are as follows: Weigh accurately an amount of the sample estimated to contain about 0.30 Gm. mercury and transfer to a 500-cc. Kjeldahl flask.

---

* Presented before the Scientific Section, A. PH. A., Richmond meeting, 1940.

† From the Control Laboratory of The Columbus Pharmacal Company, Columbus, Ohio.

Wash down into the flask with 25 cc. of hydrochloric acid and 50 cc. of water, then add 8 Gm. of zinc (C. P. 10 mesh). Shake the flask until the amalgam which adheres together has been broken up into a granular form. Add an additional 2 Gm. of zinc and heat on a steam bath, shaking frequently until the solution becomes clear and colorless. Remove about 2 cc. of the supernatant liquid and saturate it with hydrogen sulfide; it should remain colorless. If, however, a brownish tinge develops (indicating the presence of mercuric salts), continue the digestion until the solution shows no reaction with hydrogen sulfide.

When all the mercury is reduced, wash the amalgam by decantation until the washings no longer show a reaction with silver nitrate solution. In the event that fragments of the amalgam are floating on the surface of the liquid, add a few cc. of alcohol to break the surface tension.

The amalgam is now dissolved by treating with a mixture of 25 cc. concentrated nitric acid and 100 cc. of water. If the reaction becomes too violent, the flask should be cooled by immersing in cold water. When the zinc has been dissolved out of the amalgam and the reaction has moderated, add 20 cc. more of concentrated nitric acid and heat on the steam bath until all the mercury is in solution; then add 20 cc. more of concentrated nitric acid and heat for 30 minutes on a steam bath. The solution should at this time be clear and colorless.

Dilute the solution to 500 cc. in a volumetric flask and after thorough mixing, remove about 10 cc. and add 4–5 drops of dilute hydrochloric acid. The solution should remain clear; if, however, mercurous salts are present (indicated by the turbidity of the solution), a new assay must be started using more nitric acid and a more prolonged heating time to insure complete oxidation.

Titrate 100 cc. of the above solution with 0.1*N* Potassium Thiocyanate V.S. using 2 cc. of ferric ammonium sulfate T.S. as indicator.

Each cc. 0.1*N* Potassium Thiocyanate V.S. is the equivalent of:

Mercuric Chloride, $HgCl_2$	0.013576 Gm.
Mercuric Iodide, HgI_2	0.02272 Gm.
Mercury, Hg	0.01003 Gm.

Note: (1) If the original sample contains mercurous salts, it should be oxidized with bromine before proceeding with the assay. (2) If the original sample contains sodium bicarbonate as in certain germicidal tablets of mercuric iodide, diluted hydrochloric acid should be added until there is an excess equivalent to about 25 cc. (Using ten tablets, each containing mercuric iodide $^3/_8$ gr., potassium iodide $^3/_4$ gr. and sodium bicarbonate 14 gr.)

The method has been found useful in the assay of Ammoniated Mercury Ointment U. S. P. and in ointments containing ammoniated mercury and zinc oxide in combination. In this case place 15 Gm. of the ointment, accurately weighed, in a 200-cc. beaker, warm slightly to soften the ointment and while stirring add 50 cc. ether and stir the mixture until the ointment base is dissolved. Transfer to a separatory funnel, washing the beaker with ether and diluted hydrochloric acid (10-cc. portions) until the ointment is completely transferred. Shake the mixture vigorously until all the inorganic compounds have been dissolved. Filter the aqueous layer into a 500-cc. Kjeldahl flask and wash the remaining ethereal solution with several portions of distilled water until the last washing produces no turbidity with silver nitrate T.S. Add the zinc to the acid solution in the Kjeldahl flask and proceed as outlined in the general method.

SUMMARY

In our hands this procedure has been found to yield accurate results and is more rapid than gravimetric methods. It has been successfully used in the assay of antiseptic tablets of mercuric chloride where the presence of organic dyes may lead to erroneous results if the sulfide precipitation method is employed. Other applications are evident and will be developed as opportunity arises.

Note on Philippine Turtle Oil*

By Pura Villarica and Patrocinio Valenzuela†

"A giant leathery sea turtle, known in science as *Dermochelys schlegeli* (Garman), was recorded in Philippine waters for the second time when Lt. Col. Zerbee of the U. S. Army shot the reptile in Tayabas Bay, off the coast of Lucena on March 26, 1939. The monster was brought to Manila by the S.S. *Masbate* on which the colonel was a passenger when he sighted the reptile, which had some pilot fish, or remora, attached to its back. The dorsal shield or carapace of the animal measured 194 centimeters from tip to tip and the whole animal weighed about 300 kilograms. Leathery turtles are known to attain a length of about 320 centimeters and a weight of about 500 kilograms. The turtle is known to be dis-

---

* Presented before the Scientific Section of the American Pharmaceutical Association, Richmond meeting, 1940.

† College of Pharmacy, University of the Philippines.

tributed between the tropics, but is scarce wherever it occurs. It is of no great commercial value, except for the oil, which is highly prized for use in cosmetics.

"The first recorded evidence of the presence of these huge reptiles in Philippine waters is a stuffed specimen exhibited in the old Ateneo de Manila. This specimen is said to have been caught in Manila Bay.

"Immediately upon arrival in the City of Manila, Lt. Col. Zerbee presented the dead turtle to Dr. Canuto Manuel of the former Division of Fish and Game Administration of the Bureau of Science, now of the Division of Fisheries of the Department of Agriculture and Commerce of the Commonwealth of the Philippines. After retaining the huge shell, Dr. Manuel offered to us the carcass for the extraction of turtle oil."[1]

As is well known in pharmacy, turtle oil has recently come into prominence in the cosmetic industry. Hence, the kind offer of Dr. Manuel has afforded us an opportunity to study the properties of Philippine turtle oil.

EXPERIMENTAL

Extraction of Oil.—To a small quantity of the fatty tissues of the turtle a little water was added, and then the mixture was heated until all available fats were extracted. A small amount of boric acid was added to the fatty oil thus obtained.

PROPERTIES OF THE OIL

The liquid fatty oil at 28° C. is lemon-yellow in color, with marked fishy odor. It is slightly soluble in cold alcohol, sparingly soluble in hot alcohol and ethyl acetate and very soluble in ether, chloroform, benzene, carbon disulfide and acetone. Other properties[2] follow:

Congealing point	6.03° C.
Refractive index (at 34° C.)	1.4664
Specific gravity (at 32° C.)	0.9222
Acid value	0.07 mg.
Iodine value	82.2
Saponification value	198.5
Unsaponifiable matter	2.46%

When examined five months later on September 23, 1939, after standing throughout at laboratory temperature in Manila, P. I., the same oil gave the following values:

Specific gravity at 30° C.	0.9143
Refractive index at 29° C.	1.4689
Acid value	0.105 mg.
Iodine value	106.2
Saponification value	227.7

COLOR TESTS

1. Color Test Given in "Turtle Oil Facts"(1).—One drop of concentrated HNO_3 was allowed to run down the side of a small evaporating dish to come in contact with a small quantity of turtle oil. It should have a brownish coloration—first a tan color and then a brown one was produced. No violet or lavender color was developed.

2. Color Test for Cod Liver Oil as Given in the U. S. P. XI, p. 261.—A solution of 1 drop of the cod liver oil in 1 cc. of $CHCl_3$ when shaken with 1 drop of conc. H_2SO_4 acquired a violet-red tint gradually changing to reddish brown. When this color test was tried for the turtle oil, 3 drops of the oil in 1 cc. of $CHCl_3$ were used instead of 1 only. A slightly violet-red tint was obtained, but it readily disappeared and turned to a slightly brown color.

TESTS FOR VITAMINS

1. Vitamin A.—Antimony trichloride test: Antimony trichloride washed with $CHCl_3$ and dried is dissolved in $CHCl_3$ to make a 30% solution (weight in volume). After standing, the clear solution is decanted and placed in a burette. The oil to be tested is dissolved in $CHCl_3$ (20%), and to 0.2 cc. delivered from a 1-cc. burette, 2 cc. of the antimony trichloride solution is added. The liquid is at once transferred to a cell and its blue color intensity is measured against standard glasses in a Lovibond tintometer.

The test was made for the turtle oil, cod liver oil containing vitamin A and rice oil also containing vitamin A.

In the last two a pale blue fluorescence was observed, which disappeared at once and developed into a purplish brown color.

The rice oil attained, as final color, a purplish red.

2. Test for Vitamin B_1[3].—A 1:10 dilution of the oil in alcohol was made and the latter tested for its vitamin B_1 content using the McCollum and Prebluda qualitative test.[4]

No pink color was developed. This test shows that the oil does not contain vitamin B_1.

---

[1] Data furnished by Dr. Canuto Manuel, formerly of the Division of Fisheries, now of the Division of Natural History Museum, Department of Agriculture and Commerce, Commonwealth of the Philippines.

[2] Analyzed in April 1939.

[3] The test for vitamin B_1 was made owing to various requests made in our laboratory for the detection of vitamin B_1 in some fixed oils.

[4] Private communication from Dr. E. V. McCollum. Also see McCollum, E. V., and Prebluda, H. A., *Science*, 84 (1936), 433; Manalo, G. D., Concha, J., and Valenzuela P., paper read at the U. S. P. Revision Conferences, May 13, 1940.

PREPARATION OF TURTLE OIL CREAMS

The following formulas were tried:

Formula A	
Turtle oil	50
Almond oil	27
Lanolin	15
Beeswax	8

Formula B	
Turtle oil	30
Almond oil	46
Lanolin	16
Beeswax	8

Both formulas gave creams which were pale yellow and soft, the odor of lanolin being well marked in both, notwithstanding the previous addition of perfume oil.

To prepare a cream with better consistency than that of the formulas previously prepared, the following was devised:

Turtle oil	15 Gm.
Almond oil	20 Gm.
Lanolin	8 Gm.
Beeswax	10 Gm.
Borax	0.02 Gm.
Distilled water	10 cc.

The cream obtained had, besides a better consistency, a creamy opaque appearance, although the odor of lanolin still persisted. It had a paler color.

The following formula was also tried:

Turtle oil	8 Gm.
Beeswax	20 Gm.
Paraffin wax	15 Gm.
Mineral oil	80 Gm.
Borax	2 Gm.
Distilled water	50 Gm.
Perfume oil	1.5 Gm.

The cream thus prepared had a consistency suitable for the Philippine climate. It is creamy, opaque and white.

A study of the compiled data on turtle oils especially with reference to the data given by Brown (1) shows that Philippine turtle oil compares favorably with the turtle oils obtained in other countries.

SUMMARY

A liquid fatty oil was obtained from a Philippine turtle. Its physical and chemical properties were studied. Its behavior under some color tests, under the antimony trichloride test for vitamin A and under the McCollum and Prebluda test for vitamin B_1 was observed. The results of the analysis show that the oil examined compared favorably with turtle oils obtained in other countries. Using some of the formulas given in the literature on turtle oil, creams were prepared from the Philippine sample.

REFERENCE

(1) Brown, F. W., *Drug and Cosmetic Ind.*, 32 (1933), 211.

Studies on Viburnum

IX. The Pharmacognosy and Pharmacology of Viburnum Alnifolium*

By Heber W. Youngken† and James C. Munch‡

During the summer of 1939, while engaged in field work in the southern United States, the senior author ascertained that large amounts of the bark of the Hobble-bush, *Viburnum alnifolium* Marsh., were being collected in the southern Blue Ridge district and marketed as "Southern Cramp Bark" and as "Cramp Bark." Later, it was ascertained that this practice has been going on for some time, the bark being admixed occasionally by collectors with bark offered to dealers as Black Haw Tree Bark and frequently substituted for genuine Cramp Bark or Viburnum Opulus, N. F. Since certain pieces of these barks bear a striking superficial resemblance one to the other, it is not strange that this substitute has eluded identity.

The chief purposes of this investigation were to study the physical characteristics, histology and pharmacology of the stem bark of *Viburnum alnifolium* and to develop means of distinguishing it from genuine Cramp Bark.

EXPERIMENTAL

Materials and Methods.—The materials used in the pharmacognostical portion of this investigation consisted of representative parts of plants of *Viburnum alnifolium* gathered on Mt. Mitchell, N. C., and in Aroostook Co., Maine, and of representative parts of plants of *Viburnum opulus* var. *americanum* gathered in the Arnold Arboretum, Jamaica Plain, Mass., and in Aroostook Co. and Orono, Maine, by the senior author, several samples of bark labeled

* Presented before the Scientific Section of the AMERICAN PHARMACEUTICAL ASSOCIATION, Richmond meeting, 1940.

† Professor of Pharmacognosy and Botany, Massachusetts College of Pharmacy.

‡ Professor of Pharmacology Temple University, School of Pharmacy, Philadelphia, Pa.

"Cramp Bark" but which, upon comparison with authentic material proved to be *Viburnum alnifolium* stem bark, and authentic commercial Cramp Bark collected near Falmouth and Farwell, Michigan.

The shrubs were studied in the field and representative portions of them, such as leaf and flowering or fruiting branches and segments of stems of different levels from tip to base, removed and partly pressed and mounted on herbarium sheets and partly preserved in alcohol for future laboratory study.

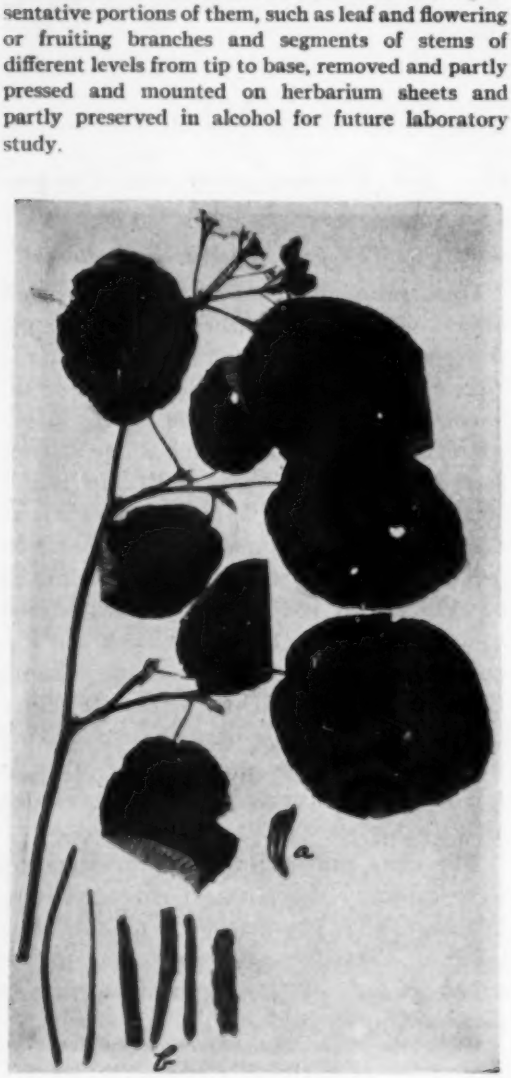


Fig. 1.—*Viburnum alnifolium*. Leaf and Fruiting Branch Showing Winter Buds. Separated Winter Buds (*a*), and Bark of Stem (*b*).

Authentic bark was also collected in the field from both of these species, partly preserved in 95% alcohol and partly dried. All specimens were labeled at the spot of collection.

The physical characteristics of the stem barks together with their histology were studied in the laboratory. Transverse, radial-longitudinal and tangential-longitudinal sections were cut from stem barks removed at different levels from twigs to bases of stems. Some of these were mounted in water, chloral hydrate solution, phloroglucin-HCl, cyanin solution and ferric chloride test solution while others were stained with safranin and fast green, dehydrated, cleared and mounted permanently in Canada balsam, preparatory to microscopical examination.

The powdered bark material discussed in this paper was ground from bark collected from nature by one of us (H. W. Y.).

Several pounds of botanically authenticated *Viburnum alnifolium* stem bark were forwarded to Dr. J. C. Munch to be used in his pharmacological studies.

Description of Viburnum Alnifolium.—*Viburnum alnifolium* Marsh., is a beautiful shrub with glossy, purple to purplish brown twigs, large, orbicular to broadly ovate, dark green leaves with short acuminate summits, cordate or sub-cordate bases and irregularly serrulate to denticulate margins. They take on a deep claret to reddish purple color in autumn. It bears compound cymes of large, white, neutral, marginal flowers and smaller, greenish, central, fertile flowers. The flowers appear in May and June and are succeeded by ellipsoidal, to broadly ellipsoidal drupes about 8 mm. in length which are at first coral-red, later becoming purple-black. The winter buds, some of which we have found in the marketed drug, are large, tomentose and cinnamon-brown. They are devoid of scales and their hairs are stellate in character. The leaves exhibit pinnate-reticulate venation and are densely pubescent beneath, especially on the veins.

Distribution.—*Viburnum alnifolium*, also known as Hobble-bush, American Wayfaring-tree, Witch Hobble and Moosewood, is distributed widely in moist woodlands from New Brunswick and Maine westward to the interior of Ontario and Michigan and southward into the mountains of Virginia, North Carolina, Tennessee and Kentucky. In the northern states it often occurs in the same general regions where *Viburnum opulus* var. *americanum* abounds.

Now, *Viburnum opulus* var. *americanum* (Mill.) Ait., the Cranberry-tree or High Bush Cranberry, which yields the genuine Cramp Bark is distinctly a northern shrub and does not occur in the wild state below the Mason and Dixon line. In Gray's "Manual of Botany" its distribution is given as along streams and in woods from Newfoundland and eastern Quebec to British Columbia south to New Jersey, Pennsylvania, Michigan, Wisconsin and N. E. Iowa (1). It occurs in LaGrange Co., Indiana, and is very abundant in Maine and in certain sections of Michigan.

Physical Characteristics of Stem Bark.—The bark on the young twigs of *Viburnum alnifolium* is externally purplish to purplish brown, smooth and polished with scattered circular to oval, raised lenticels and less than 0.5 mm. in thickness when dried. The fracture of its outer and middle bark is short, that of the inner bark, short-fibrous, the fractured surface showing projections of fibers in the inner bark and this region frequently separating

in the process of breaking and exposing the greenish middle bark. The inner surface of this young twig bark is pale brown and longitudinally striate on pieces of entire bark, but many pieces of commercial young bark are devoid of inner bark and so exhibit the green color of the middle bark.

The dried bark from older portions of the stem varies on its outer surface from purplish brown, through dark brown to blackish brown. Some pieces show grayish foliaceous lichens, some light brown lenticels, while others exhibit irregular longitudinal fissures, cracks and ridges. As this bark becomes older, the cork becomes cracked in irregular longitudinal fashion and through these cracks the greenish to light-colored subjacent tissue is to be noted. It also becomes scaly, the scales representing dead cork tissue in the process of exfoliation.

The fracture of the older bark is short in the outer bark zone and fibrous in the inner bark. The cork is readily detachable in areas on some of the pieces, while other pieces exhibit partly detached outer, middle and inner zones of bark. The inner surface of older pieces of bark varies from pinkish brown to pale brown and is finely longitudinally striate to nearly smooth; that of many pieces of commercial bark exhibited a whitish, adherent wood

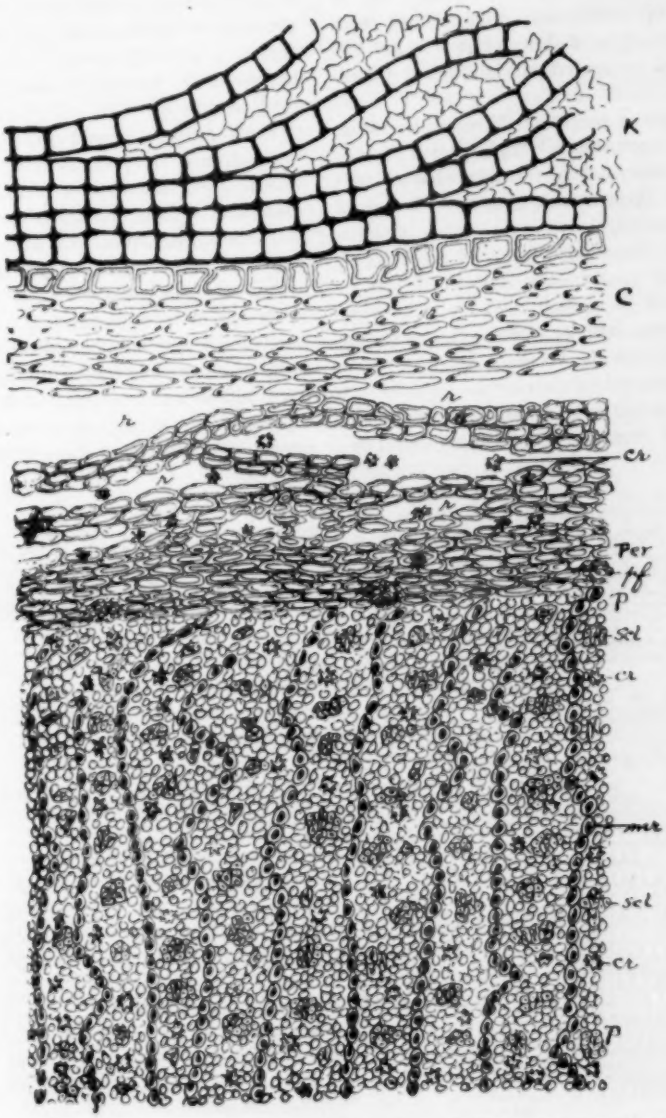


Fig. 2.—Transverse Section of Portion of Older Stem Bark of *Viburnum alnifolium*, × 85.

K, cork, consisting of thin- and thick-walled cork cells which alternate in places; *C*, cortex; *r*, rifts; *cr*, rosette aggregates of calcium oxalate; *Per*, pericycle; *pf*, pericyclic fibers; *P*, phloem; *scl*, bast fibers; *mr*, medullary ray.

Admixed with the average samples of commercial bark examined were twigs of purple to purplish brown color which possessed a large, white pith and also occasional naked winter buds. The latter were up to 2.3 cm. long, cinnamon-brown to purplish brown in color and covered with a rufous tomentum.

Toward the base of older stems of this shrub the outer bark is grayish black scaly, with the cork separated by short fissures into numerous small, polygonal plates.

The odor of the bark is slight when dry but characteristically valeric acid-like when the bark is boiled with water or triturated with phosphoric acid. The taste is bitter and somewhat astringent.

Histology of the Stem Bark.—Sections cut at different levels in succession, commencing with the young twig bark and descending toward the base of the stem presented the following microscopic structure:

No. 1.—Transverse section 0.75 mm. in radial diameter. (First-year growth.) This section showed an epidermis of somewhat rectangular-shaped cells in the process of disintegration, 5 or 6 layers of tangentially elongated, wavy cork cells, beneath which were noted interrupted areas of loose, open tissue representing young cork and phellogen in the process of cork formation. These cells were large and more or less radially elongated, the young cork cells with thin, colorless walls. Between these areas the cells appeared meristematic. There followed a relatively broad zone of cortex whose outer region consisted of from 10 to 12 layers of tangentially elongated, porous parenchyma tissue, some of the cells of which contained starch, others rosette aggregates of calcium oxalate. The inner zone of cortex of a nearly equal magnitude was composed of larger-celled parenchyma with similar contents. Rifts occurred in both zones. Beneath the cortex a narrow zone of pericycle was noted consisting of starch- and crystal-bearing parenchyma through which coursed numerous pericyclic fibers forming an interrupted arc. These were arranged in small groups of 2 to 9 fibers and as occasional isolated fibers. In transverse view they were mostly elliptical in outline, with walls averagely considerably thicker than lumen. Most of them possessed non-lignified walls, a few showing a beginning of lignification. Beneath this region occurred a phloem, up to 171μ in radial diameter traversed by nearly straight medullary rays of 1 to 2 cells in width.

Each phloem strand was composed of phloem parenchyma, sieve tubes and companion cells.

No. 2.—Transverse section 0.95 mm. in radial diameter. This section showed fully developed lenticels. The epidermis was adherent in shreds. The cork consisted of several layers of tangentially elongated cork cells of wavy contour and brown walls which were suberized. Beneath this was a phellogen which had cut off on its outer face a zone of large, clear, thin-walled, young cork cells which in some places was but one cell in breadth, in others, especially in the region of lenticels, 3 or 4 layers broad. On the inner face of the thin-walled cork

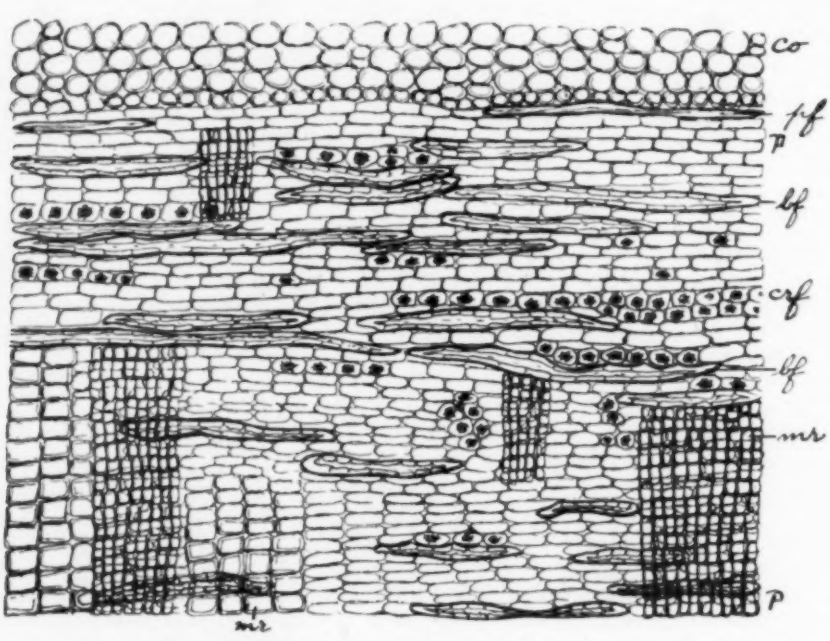


Fig. 3.—Longitudinal-Radial Section of Inner Cortex, Pericycle and Phloem, Old Stem Bark of *Viburnum alnifolium*, × 85.

co, cortex; *pf*, pericyclic fibers in pericycle; *P*, phloem; *bf*, bast fibers; *crf*, crystal fibers containing rosette aggregates of calcium oxalate; *mr*, medullary rays.

zone there occurred 1 to 2 layers of tangentially elongated, brown cork cells with suberized walls. The cortex and pericycle were similar to section 1 in aspect, excepting that here and there a pericyclic fiber showed a beginning of lignification as evidenced by the pink color imparted to the peripheral portion of its wall. The phloem region resembled No. 1 excepting for the appearance of a few phloem fibers in its outer region. These were non-lignified.

Nos. 3 and 4.—Transverse sections 0.63 mm. to 1 mm. in radial diameter. These were similar to the last but with more epidermis exfoliated. No. 4 showed a slightly broader phloem.

No. 5.—Transverse section 1.12 mm. in radial diameter. Exfoliation of outer cork layers had begun. More rifts were evident in the cortex and pericycle. The phellogen had laid down in places a second zone of open, thin-walled and radially elongated cells beneath the second zone of thick-walled, brown cork cells. Lignification had progressed in the bast fibers.

No. 6.—Transverse section 1.135 mm. in diameter. The periderm showed alternating zones of thick-walled, brown and thin-walled, larger and open-looking cork cells. The cortex showed an increased number of rifts. The phloem was slightly broader with an increased number of bast fibers the latter showing increased lignification. The medullary rays occurred in convergent groups. The outer cork layers were partly exfoliated.

No. 7.—Transverse section 1.520 mm. in radial diameter. The periderm showed considerably more loose, thin-walled, suberous tissue. It contained several alternating zones of brown and colorless cork cells, the colorless zones being mostly broader than in the preceding sections. The cortex was broader owing to the phellogen having cut off secondary cortex. The radial diameter of the phloem was 320μ. The phloem showed an increased number of bast fibers, some of these being in large, irregular, tangentially arranged groups. Some of the phloem rays were wavy in character. Rifts were numerous. Druses appeared more numerous than in the previous sections.

No. 8.—Similar to No. 7 but showing more exfoliation of periderm. This section was 1.55 mm. in radial diameter.

No. 9.—Transverse section 1.2 mm. in radial diameter. This section was cut through a lower level of the bark than the preceding, its smaller diameter being accounted for by the loss of considerable of its cork through exfoliation. Passing from outer to inner margin of section, the following was observed: An outer zone of collapsed, thin-walled cork cells, a broad zone of several layers of thick-walled cork cells of dark brown aspect, interrupted zones of thin-walled, somewhat radially elongated cork cells, a phellogen, a broad cortex, showing many rifts, a narrow pericycle with an increased number of fibers arranged in interrupted groups, a few isolated, a broader phloem with convergent groups of medullary rays, some of the rays being straight, others curved in their course. The phloem patches contained scattered groups of bast fibers most of which possessed thick non-lignified walls, a scattered few showed lignification. A number of phloem patches contained secondary phloem rays. Rosette aggregate crystals of calcium oxalate were abundant in the parenchyma of the section.

No. 10.—Transverse section of Old Stem Bark, 2.88 mm. to 3 mm. in radial diameter.

1. Periderm of many alternating layers of thin-walled and thick-walled cork tissue and a phellogen. The thick-walled cork cells were orange to brown in color and more or less collapsed.

2. Secondary cortex of up to 10 layers of collenchyma, the cells containing either starch grains, druses or resinous substance. The druses were up to 40μ in diameter.

3. Primary cortex of several layers of starch- and crystal-containing parenchyma, the cells tangentially elongated. Several rifts occurred in the cortical zones.

4. A pericycle consisting of a narrow zone of starch- and crystal-bearing parenchyma in which occurred numerous groups of pericyclic fibers with lignified walls.

5. Phloem, consisting of a very broad zone (1440μ in radial diameter) separated into many oblong phloem patched by phloem rays, the latter 1 to 2 cells in width. A large number of bast fibers with lignified walls and occurring either isolated or in groups of from 2 to 12 were to be found in every phloem patch.

Sections mounted in ferric chloride solution showed abundant tannin.

Radial-longitudinal section of Old Stem Bark. In this section the collenchyma cells appeared rounded to only slightly elongated and showed irregular lumina and walls of irregular thickness. Some of the pericyclic fibers were slightly lignified but others non-lignified. They possessed very thick walls, narrow lumina and attenuated, often obtuse ends and were up to 22.8μ in width. Some of them were wavy walled. The walls of these fibers were up to 11μ in thickness. Numerous rosette crystals of calcium oxalate and crystal fibers containing rosette crystals were found in the parenchyma of the cortex and phloem.

The medullary rays, crossing areas of the phloem, possessed cells with beaded, porous walls, some of the walls being lignified. The bast fibers were numerous in the phloem. Those measured were up to 38μ in breadth. They showed great irregularity in respect to their walls and lumina. The walls varied considerably in thickness, degree of lignification and in contour. Some were wavy and tuberculate, others lobed. Their ends varied, some being curved or hooked, others acute, rounded or lobed. Occasional stone cells were met with in some of the sections. These usually occurred in smaller groups than in *Viburnum opulus* or as isolated elements.

Tangential-longitudinal section of Old Bark. In this section the medullary rays were 1 to 2 cells in width. The great irregularity in contour and thickness of the walls and lumina of the fibers appeared most striking and diagnostic. Some of them were wavy toothed or wavy, and their ends varied from acute, obtuse, truncate to lobed.

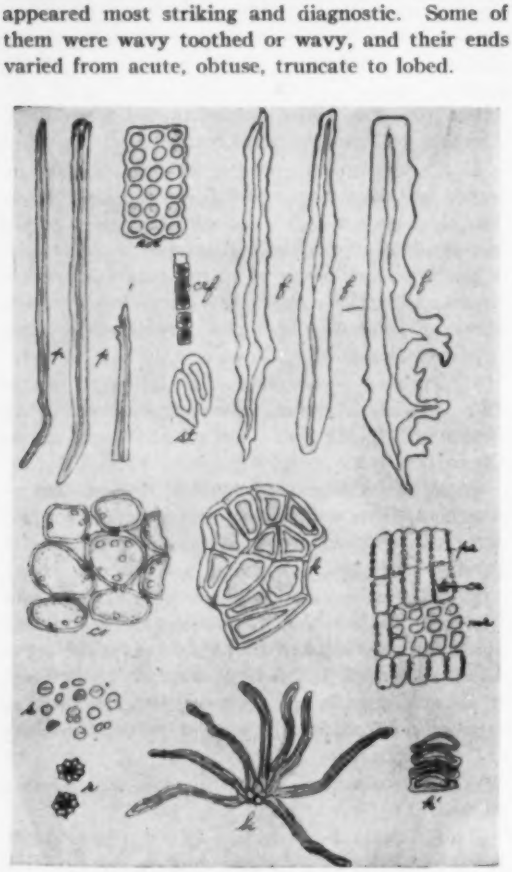


Fig. 4.—Powdered *Viburnum alnifolium* Stem Bark. *p*, pericyclic fibers; collenchyma from cortex; *crf*, crystal fiber; *st*, stone cells; *f*, phloem fibers; *co*, cortical parenchyma; *pa*, phloem parenchyma; *cr*, rosette aggregate crystal of calcium oxalate; *mr*, portion of phloem medullary ray in longitudinal radial view; *k*, cork in surface view; *k'*, cork in traverse view; *s*, starch grains; *h*, aggregate hair from epidermis of bud of *V. alnifolium* which is frequently found in the powdered stem bark.

Powdered Viburnum Alnifolium Stem Bark.—Light brown. Starch grains mostly simple, a few from 2- to 3-compound, the individual grains mostly under 8μ in diameter and of spheroidal, oval or plano-convex shape with central cleft hilum; numerous fragments of starch- and crystal-bearing parenchyma and collenchyma, an occasional starch grain up to 15μ; with slightly excentric, cleft hilum; numerous rosette aggregates of calcium oxalate up to 40μ in diameter; fragments of cork tissue of brown or orange color and of irregularly polygonal or rectangular cells with brown, amorphous contents, the cork often adherent to subjacent collenchyma; numerous fragments containing sclerenchyma fibers having narrow, irregular lumina and with thick, non-lignified to lignified, frequently irregular, wavy walls, the latter from about 4 to 12μ in thickness; an occasional stone cell or group of stone cells, the latter with broad lumina.

Distinctions between Viburnum Opulus and Viburnum Alnifolium Stem Barks.—In a paper by one of us (2) published in THIS JOURNAL in 1932, the physical characteristics and histology of the stem bark and root bark of *Viburnum opulus* var. *americanum* was discussed at some length.

Since that time, considerable additional material has been examined representing stages in the development of these barks not previously studied. The results of these additional studies in so far as they apply to the practical methods of distinguishing the two stem barks here discussed will be incorporated with the relevant portion of the earlier paper in distinguishing between Viburnum Opulus, N. F. and Hobble-bush stem bark. The chief differences follow.

The outer surface of dried young twig bark of *Viburnum opulus* var. *americanum* is purplish to purplish brown with a finely fibrous fracture and its surface is polished, but the average commercial sample of young bark is collected back of the first or second node of the stem and this varies from gray to grayish brown to greenish yellow or greenish brown and is irregularly longitudinally wrinkled, often showing a few slightly raised lenticels. Such bark occurs in thin pieces.

The outer surface of the stem bark of the Hobble-bush or *Viburnum alnifolium* as found on young twigs and on most of the thinner pieces of this commercial bark is purple to purplish brown and glossy and tends to possess more lenticels. Both barks show a greenish phelloderm, where abraded.

The fracture of most thin pieces of commercial authentic *Viburnum opulus* is short and weak, exhibiting few or no projecting fibers, whereas that of *Viburnum alnifolium* is short in the outer bark and fibrous in the inner bark, and it frequently separates into layers on breaking.

Dried older stem bark of *Viburnum opulus*, as represented by thicker pieces shows an outer surface which varies from gray through grayish brown or grayish black to black (when abraded, greenish to brown) and is longitudinally wrinkled, finely fissured or scaly, whereas dried older stem bark of *Viburnum alnifolium*, represented by the thicker pieces of this bark, has an outer surface which varies from purplish brown, dark brown to blackish brown (when abraded, greenish to reddish brown or cinnamon-brown) and either shows numerous, irregular, longitudinal cracks through which light-colored subjacent tissue is evident or, on very old bark, shows numerous, small, irregularly square or rectangular cork plates (scales) separated by narrow fissures.

The fracture of old *Viburnum opulus* is short and brittle, whereas that of old *Viburnum alnifolium* stem bark is short in the outer bark and splintery-fibrous in the inner bark.

Under the microscope thin transverse sections of most pieces of *Viburnum opulus* stem bark may readily be distinguished from similar sections of stem bark of *Viburnum alnifolium* (1) by the former having cork composed of alternating layers of cells of two kinds, *viz.*, a layer of cells whose inner walls are lignified or both outer and inner walls lignified and a layer of cells having non-lignified walls, whereas the latter show zones of brownish, compressed, suberous-walled cells, alternating with thin, colorless-walled layers of cells.

Whereas some sections of thinner pieces of *Viburnum opulus* stem bark show few or no fibers in the pericycle, fibers are numerous in thinner pieces of the stem bark of *Viburnum alnifolium* both in the pericycle and the phloem.

Sections of old bark of the stem of *Viburnum opulus* exhibit groups of stone cells in the phloem

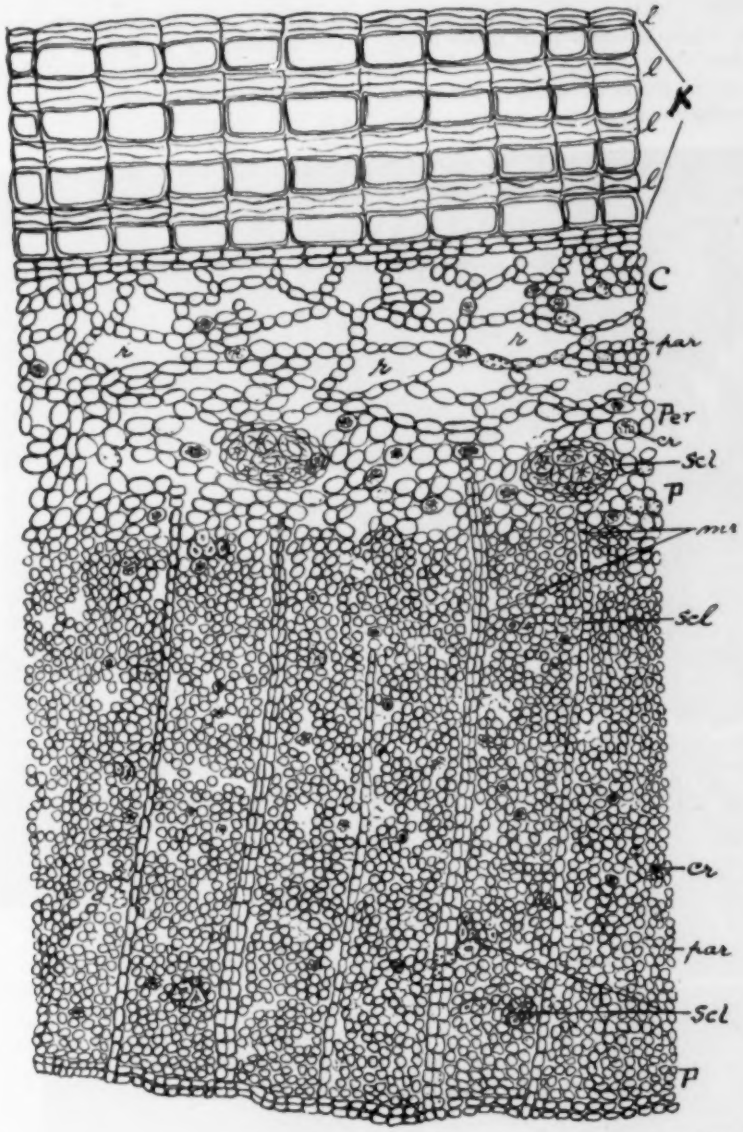


Fig. 5.—*Viburnum Opulus* var. *americanum*. Cross Section of Older Stem Bark, × 75.

K, cork, consisting of layers of cells possessing lignified inner and frequently outer walls alternating with layers with non-lignified walls; *l*-cork cells with lignified outer and inner walls; *C*, cortex; *Per*, pericycle, *P*, phloem; *r*, rifts or spaces surrounded by parenchyma which are abundant in the cortex and pericycle and which also occur in the phloem of this bark; *par*, parenchyma; *Scl*, sclerenchyma; *cr*, druses; *mr*, medullary rays.

which are not infrequently widely scattered, whereas sections of similarly aged bark of the stem of *Viburnum alnifolium* show numerous, closely set groups of bast fibers in the phloem and only an occasional stone cell or small group of stone cells.

Pharmacology.—Several lots of *Viburnum alnifolium* which had been identified by one of us (H. W. Y.) were made into fluidextracts by the N. F. VI method for pharmacological study. To avoid any interfering action by alcohol, these solutions were evaporated over a low heat on an electric hot plate to about $^1/_4$ of their original volume, then restored to the original volume with water immediately before use.

A series of tests on the isolated guinea-pig uterus showed that this dealcoholized solution was a uterine sedative, and physiologically antagonized the oxytoccic action of posterior pituitary extract. The stimulant action of pilocarpine was also antagonized. Some evidence of tetany developed after prolonged exposure to strong solutions of *Viburnum alnifolium*.

Figure 6 shows a typical response obtained by the intravenous injection of dealcoholized *Viburnum alnifolium* to a dog anesthetized with morphine and chloretone, and in which the vagus nerves were blocked with atropine. The technique used is essentially that for the assay of epinephrine, outlined in U. S. P. XI, except that curare is not used. The injection of 10 gamma of U. S. P. Standard Epinephrine produced the typical pressor response, the pressure rising from the pre-injection level of 102 mm. to 142 mm., or 40 mm. at 16.22. The

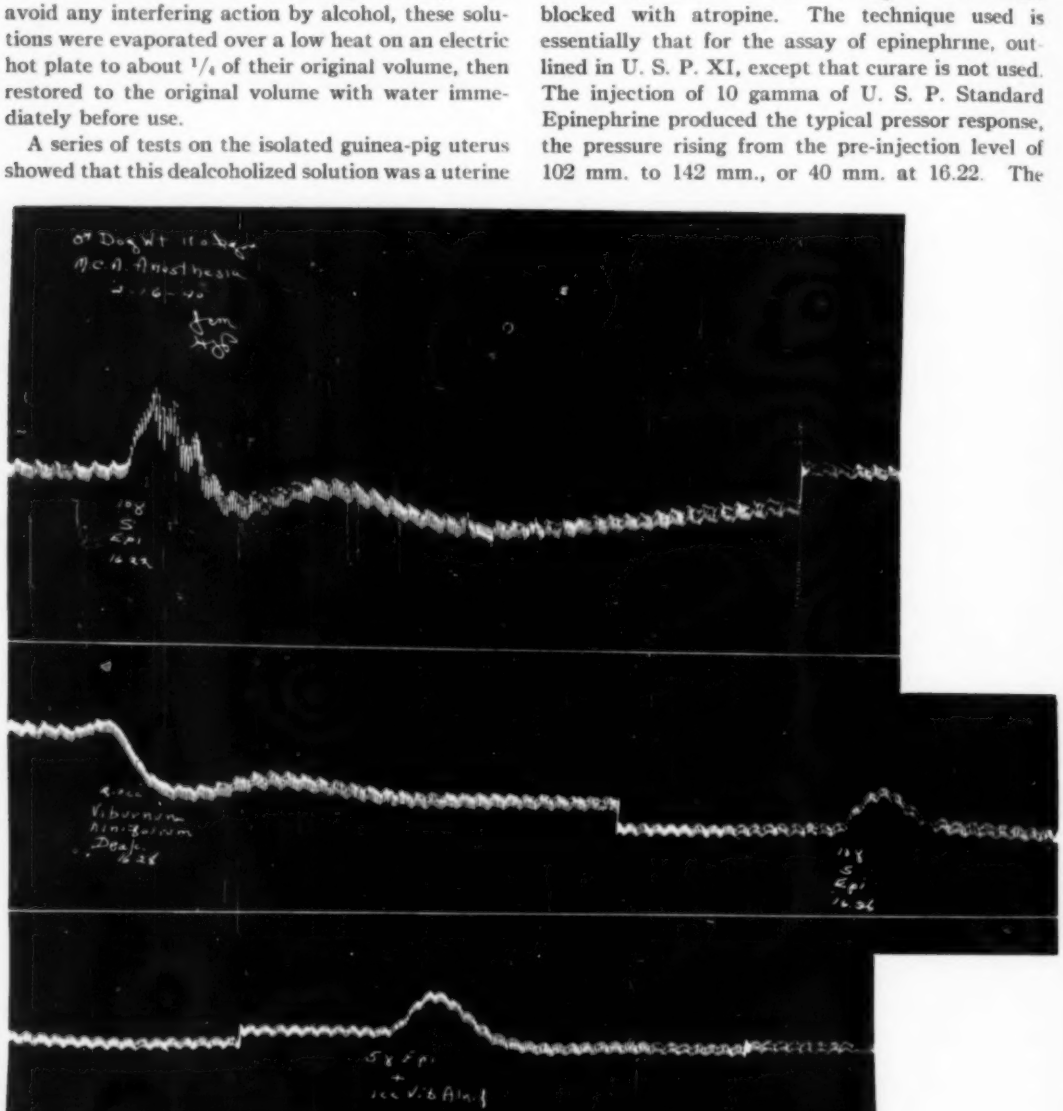


Fig. 6.—Effect of Dealcoholized Fluidextract of Viburnum Alnifolium on the Blood Pressure of an Anesthetized Dog. Note Toxic Effect and Death.

post-injection level of 104 mm. decreased to 70 mm. following the injection of 2 cc. of dealcoholized *Viburnum alnifolium* intravenously at 16.28 and this pressure was maintained. At 16.36 the blood pressure was 50 mm. and the injection of 10 gamma of epinephrine caused a rise to 72 mm., or an increase of 22 mm.

At 16.40 the blood pressure was stabilized at 54 mm.; the injection of a mixture of 5 gamma of epinephrine with 1 cc. of dealcoholized Fluidextract Viburnum Alnifolium caused a rise to 72 mm., or an increase of 18 mm. in pressure. Since this is less than the rise produced by twice the dose of epinephrine alone, but almost reaching the same level (18 mm. *vs.* 22 mm.) it appears that 1 cc. of this sample is equivalent to not more than 5 gamma of epinephrine, physiologically, and probably to less than 5 gamma.

At 16.43 the injection of 1 cc. of Fluidextract Viburnum Alnifolium with 2.5 gamma of epinephrine caused a rise in blood pressure from 46 mm. to 50 mm., or there was practical neutralization of pressor and depressor activity. This would suggest that 1 cc. of the Fluidextract Viburnum Alnifolium was equivalent to 2.5 gamma of epinephrine.

The further injection of 5 cc. of dealcoholized Fluidextract Viburnum Alnifolium led to death in 2 minutes. This represents a total dose of 2 plus 1 plus 1 plus 5, or 9 cc. of Fluidextract Viburnum Alnifolium to a dog weighing 11 Kg., or approximately 0.8 Gm. of *Viburnum alnifolium* bark per Kg. None of the other species of Viburnum studied have proved toxic to dogs when given in doses as large as 10 Gm. per Kg. which suggests that there is a toxic component in *Viburnum alnifolium* bark in addition to the depressor-uterine sedative constituent. Further studies are being conducted in the connection.

SUMMARY AND CONCLUSIONS

1. The stem bark of the Hobble-bush, *Viburnum alnifolium* Marsh., has been frequently substituted for Viburnum Opulus, N. F. and used to adulterate Black Haw Tree Bark.

2. A description is given of the materials used in this investigation and of the methods employed therein.

3. The shrub *Viburnum alnifolium* is described. Its winter buds and segments of its young, purple stems occurred in many samples of the commercial *Viburnum alnifolium* bark which has been marketed by collectors in the southern United States as "Southern Cramp Bark" and as "Cramp Bark."

4. The distribution of *Viburnum alnifolium* is compared with *Viburnum opulus* var. *americanum*. It is shown that *Viburnum opulus* var. *americanum* does not occur in the wild state in the southern United States.

5. The physical characteristics of the young and old stem barks of *Viburnum alnifolium* are described.

6. A description is given of the histology of ten different thicknesses of the stem bark of *Viburnum alnifolium*.

7. The powdered substitute bark is described. Stellate hairs from the naked winter buds of the shrub yielding it occurred in this powder.

8. Macroscopic and microscopic points of difference between the younger and older stem barks of *Viburnum opulus* var. *americanum* and *Viburnum alnifolium* are elucidated.

9. The methods and results of pharmacologic studies on the stem bark of *Viburnum alnifolium* are reported. It is shown that in addition to the typical uterine sedative and depressor action of the previously studied medicinal Viburnum barks, this bark appears to contain an unknown and unsuspected toxic constituent. In the light of present knowledge, it should not be used under the same conditions as other medicinal Viburnums such as *Viburnum prunifolium* and *Viburnum opulus*.

REFERENCES

(1) Gray's "New Manual of Botany," 7th Edition (1908), page 759.

(2) Youngken, H. W., JOUR. A. PH. A., 21 (1932), 444.

Emil Fischer (1852-1919) was the second recipient of the Nobel Prize for Chemistry. He received the prize in 1902 in recognition of the extraordinary merit of his work on the synthesis of the sugars and purine compounds.

"The pleasantest things in the world are pleasant thoughts; and the great art of life is to have as many of them as possible."
—Montaigne

Marihuana Investigations. III

The Effect of Region of Growth of Hemp on Response to the Acid and Alkaline Beam Tests

By B. B. Robinson and J. R. Matchett†*

INTRODUCTION

Previous articles (1) of this series have pointed out among other findings that: (1) individual hemp (*Cannabis sativa*) plants, though of the same commercial source of seed and grown side by side, vary widely in response to the acid and alkaline Beam tests; (2) different agronomic varieties of Cannabis vary widely in number and intensity of positive responses; (3) intensity of response increases somewhat with advancing age of plants; (4) sex of plants has probably little bearing on number or intensity of responses; (5) varying fertilizer treatments of the soil have no significant influence on the number or intensity of responses.

It has been shown that neither the alkaline nor the acid Beam tests offer promise as quantitative measures of physiological potency (2). Neither test has been proved adequate as a criterion of the presence or absence of marihuana in the qualitative sense since many specimens respond to neither. Nevertheless the varying responses to the tests definitely indicate variations in the chemical make-up of the resin elaborated by the plant and are valid measures, within obvious limits, of the effects produced by the conditions studied. In the case of the alkaline test the resin constituent measured appears to be cannabidiol recently isolated by Adams (4) and his co-workers. In the case of the acid test the responsive constituent appears to be a naturally occurring combination of an unidentified aldehyde with one or more resin constituents (2).

The purpose of the present research was to study the resin content and Beam test response of plants grown under conditions differing as widely as possible with respect to rainfall, temperature, light and elevation. It will be shown that dry climate is believed to enhance response to the alkaline test but that this influence is small as compared to that of agronomic variety. In the case of the acid test it will be shown that different locations do not produce differences that are significant except when considered with dates of harvest and varieties. Results are presented to show that the resins vary in quantity with age of plant, agronomic variety and regional environment.

EXPERIMENTAL

METHODS OF TESTING

The separate hemp studies were conducted as similarly as possible except that the cultural experiments were carried out at four widely separated locations accessible for the work. The particular locations[1] were: Arlington Experimental Farm, Arlington, Virgina; Delta Experiment Station, Stoneville, Mississippi; Nebraska Experiment Substation, North Platte, Nebraska; and Wisconsin Agricultural Experiment Station, Madison, Wisconsin.

The Nebraska location represented a dry semi-arid region with an elevation of approximately 3000 feet. The rainfall during the growing season at the Nebraska station was equal to the rainfall at the Wisconsin station but the supply of stored moisture in the soil must have been materially less as may be observed by studying Table I. The regional studies in Virgina, Wisconsin and Mississippi were all under so-called humid conditions and below 1000 feet in elevation. The growth of the hemp was probably influenced to some extent by differences in photoperiodism as the Mississippi experiment was located approximately 675 miles south of the Wisconsin experiment. Differences in photoperiodism are known to influence the growth of hemp.

Seed of four single plant selections representing four strains or varieties of hemp were used in the experiments. The selections represented varieties or strains of different origin, Manchurian "A," a selection from seed of Manchurian origin which had been bred and selected for a low alkaline Beam reaction, Manchurian "B," a selection which had been bred and selected similar to "A" but not with an equal result, African"C," a selection from seed received from Tunisia, Africa, and bred and selected for low alkaline Beam response, and Kentucky "D," a single plant selection not selected in any way for the Beam test response. The Kentucky variety from which the selection was made represents a variety which had been grown in the United States for many years but originally was of Eastern Asiatic origin.

* Agronomist of the U. S. Department of Agriculture, Division of Cotton and Other Fiber Crops and Diseases.

† Chemist of the U. S. Treasury Department, Bureau of Narcotics.

[1] The writers wish to express their appreciation to Supt. H. C. McNamara, Stoneville, Mississippi; Supt. L. L. Zook, North Platte, Nebraska; and Prof. A. H. Wright, Madison, Wisconsin, for their coöperation in conducting the cultural tests.

Table I.—Climatological, Cultural and Dates of Sampling Data Pertaining to the Regional Experimental Tests

Period	Temperature—Mean Degrees, F. Wisconsin	Nebraska	Virginia	Mississippi	Precipitation—Number, Inches Wisconsin	Nebraska	Virginia	Mississippi
Sept. 1938	62.0	66.0	67.4	76.2	10.29	4.56	4.27	0.26
Oct. 1938	55.8	58.7	58.8	66.6	0.76	0.03	1.15	1.27
Nov. 1938	37.8	35.3	49.8	53.2	2.16	0.23	2.60	2.56
Dec. 1938	24.2	30.0	38.4	46.6	0.89	0.16	2.69	3.38
Jan. 1939	26.2	32.6	37.8	49.1	1.95	0.70	3.41	12.24
Feb. 1939	19.5	23.3	42.3	49.8	1.75	0.15	5.71	5.69
Mar. 1939	33.0	40.5	46.4	58.2	1.25	0.88	2.89	4.17
Apr. 1939	42.8	49.2	53.4	61.2	3.16	1.30	3.78	5.29
May 1939	62.4	65.2	68.0	71.3	1.64	2.86	0.41	3.17
June 1939	69.8	69.6	75.4	79.7	2.33	3.85	4.55	5.61
July 1939	74.8	79.9	76.2	82.5	1.64	0.14	2.01	1.97
Aug. 1939	71.3	73.4	78.8	84.4	2.61	1.53	3.22	0.51
Total year					30.43	16.39	36.69	46.12
Crop season	72.3	71.1	73.5	70.6	5.54	7.97	8.30	18.47
Elevation—approx. feet	974	3000	25	127				
Crop season								
No. days long	86	123	113	134				
Date planted	May 28	Apr. 24	Apr. 25	Mar. 9				
Date 1st sample	June 28	June 13	May 29	Apr. 20				
Date 2nd sample	July 21	July 10	July 3	May 18				
Date 3rd sample	Aug. 10 & 21	Aug. 9 & 24	Aug. 15	July 20				

The seed of each selection was divided into four lots, distributed to the four experiment stations and planted in four separate replicated randomized experimental plots. Thus, at each location there were four varieties, each planted in four replicated plots. By using the seed of single plant selections the inheritance or genetic make-up of the plants grown for a single variety at the different stations were as similar as possible. This might not have been necessary if there were available any varieties of hemp which might be considered genetically pure but it is not believed that any hemp breeding has progressed to the point that varieties are available with a genetic purity equal to that found in certain other crops as, for example, cereals.

On three occasions during the growth of the plants, vegetative samples were collected for testing. On the first occasion the plants had not made a large growth so five separate entire plants from each plot were taken. At the second and third sampling the tops were cut from five plants from each plot. At the first two samplings the sex of the plants was indeterminable but at the time of the third sampling the sex was evident. This permitted the third harvest to consist of three female tops and two male tops to represent each plot.

The planting and sampling dates are given in Table I which shows variations due to regional climatic differences. However, in planning the work an effort was made to have similarity of cultural and sampling technique. The first samples were taken 4 to 5 weeks after planting and the second samples collected about 8 weeks after planting. The third sampling was planned to correspond to a definite stage of the plants' growth, *i. e.*, the time the male plants were flowering and shedding pollen. The African "C" and Kentucky "D" were later in maturing than the Manchurian selections and this was so marked that at two of the stations separate days were used in the collection of the third samples.

All of the samples collected were air-dried and then forwarded to Washington, D. C., where weighed samples of 0.5 Gm. were prepared of each plant for testing by the Beam tests. The Beam tests were made in the laboratory of the Internal Revenue Bureau, Philadelphia, Pennsylvania. The methods used and the recording of results were similar to those described in an earlier paper (2). Separate weighed samples of 0.25 Gm. were prepared of each plant. Twenty of these samples representing the same variety at each location and at each period of harvest were bulked together and petroleum ether extractions were made upon them in the laboratory of the Internal Revenue Bureau,[3] Kansas City, Missouri. The following procedure was used:

1. Dry at 65° C. to 70° C. for 12 hours.
2. Weigh a 5-Gm. sample of dry material.
3. Extract with petroleum ether in Soxhlet apparatus for 12 hours.
4. Remove the solvent on a steam bath before a fan.
5. Heat at 100° C. for 15 or 20 minutes to remove moisture.
6. Cool and weigh the residue.
7. Calculate the per cent resin.

The methods of planting, sampling and testing allowed a statistical investigation of the results to determine the significance of differences which were

[3] The writers express their appreciation to Mr. A. J. Mottern and Mr. L. E. Dale, chemists in charge, respectively, of the laboratories at Philadelphia and Kansas City for their assistance in performing these tests.

obtained. The statistical method applied was the "analysis of variance."[2]

RESULTS

The climatological observations at the four experiment stations are shown in Table I. This table further includes data regarding the dates of planting and dates of sampling. Although the differences in mean temperature are very slight during the growing season at the different stations the growth differences were marked as may be partly shown by the data in Table I which shows the number of days elapsing between planting and the third harvest or time of maturing of the males. This represented only 86 days in Wisconsin, the most northerly station, compared with 134 days in Mississippi, the most southerly station. The same degree of growth required about 123 days in Nebraska which is long, partly due to dry conditions at the time of planting which required nearly two weeks to elaspse before the seedlings emerged.

It is difficult to correlate climatological observations with response to the Beam tests in the present experiments but the regional results lend themselves well for statistical interpretation with other variable factors.

Table II.—Actual Results Obtained in Response to the Alkaline Beam Test. Each Individual Entry in the Table under a State Is the Total Numerical Response of Five Separate Plant Tests and Represents a Single Plot of a Variety at a Location for a Particular Test Period

Variety	Virginia				Wisconsin				Nebraska				Mississippi				Variety Sub-total	Variety Grand Total
First Sampling																		
Manchurian "A"	0	0	0	0	1	0	1	0	0	2	2	3	0	0	0	0	9	
Manchurian "B"	0	0	0	0	0	5	1	0	4	2	2	2	0	0	0	0	16	
African "C"	0	0	0	0	1	0	1	0	2	0	0	2	0	0	0	0	6	
Kentucky "D"	8	6	3	4	9	4	9	8	8	14	5	10	3	4	4	2	101	
State subtotals		21				40				58				13			132	
Second Sampling																		
Manchurian "A"	1	0	0	1	2	0	0	0	6	1	3	1	0	0	0	0	15	
Manchurian "B"	3	1	0	0	2	0	0	0	3	5	3	4	0	0	0	0	21	
African "C"	0	1	0	0	0	0	0	0	3	2	0	2	1	0	0	1	10	
Kentucky "D"	17	8	8	10	7	3	5	6	15	12	9	13	8	9	11	6	147	
State subtotals		50				25				82				36			193	
Third Sampling																		
Manchurian "A"	0	0	0	2	2	0	2	3	0	0	0	1	0	0	0	0	10	34
Manchurian "B"	2	0	2	0	1	2	3	0	1	0	0	0	5	0	2	6	24	61
African "C"	0	0	0	0	1	0	0	3	3	3	5	1	0	0	1	1	18	34
Kentucky "D"	15	12	7	17	9	7	9	9	16	9	14	10	14	11	16	6	181	429
State subtotals		57				51				63				62			233	
STATE GRAND TOTALS		128				116				203				111				

Table III.—Actual Results Obtained in Response to the Acid Beam Test. Each Individual Entry in the Table under a State Is the Total Numerical Response of Five Separate Plant Tests and Represents a Single Plot of a Variety at a Location for a Particular Test Period

Variety	Virginia				Wisconsin				Nebraska				Mississippi				Variety Sub-total	Variety Grand Total
First Sampling																		
Manchurian "A"	0	4	1	5	5	5	5	0	5	1	4	5	0	0	0	0	40	
Manchurian "B"	0	0	0	3	1	5	3	1	3	0	2	3	0	0	0	0	21	
African "C"	2	1	3	3	3	2	1	3	5	5	1	0	0	0	0	0	29	
Kentucky "D"	0	1	0	4	1	5	5	4	0	3	3	2	0	0	0	0	28	
State subtotals		27				49				42				0			118	
Second Sampling																		
Manchurian "A"	3	1	2	3	6	2	4	4	5	1	3	5	2	1	5	5	52	
Manchurian "B"	1	0	2	0	0	1	1	3	4	5	4	4	1	2	3	2	33	
African "C"	4	3	5	0	10	5	6	8	10	6	7	7	5	8	5	9	98	
Kentucky "D"	3	3	5	1	4	3	4	4	2	5	2	4	1	3	1	5	50	
State subtotals		36				65				74				58			233	
Third Sampling																		
Manchurian "A"	6	6	8	11	6	7	6	5	7	6	5	5	5	4	9	6	102	194
Manchurian "B"	5	11	9	5	6	5	6	5	9	5	8	7	6	3	1	1	92	146
African "C"	8	11	13	8	10	10	9	11	14	13	12	13	14	14	18	20	198	325
Kentucky "D"	6	8	11	2	8	6	4	5	3	2	5	3	7	10	5	17	102	180
State subtotals		128				109				117				140			494	
STATE GRAND TOTALS		191				223				233				198				

Tables II and III show the actual results obtained at the four stations upon the four varieties at the three times of sampling. Previous results as mentioned in the introduction have shown the fact that sex plays little part in the response to the Beam tests. However, the results obtained at the third sampling on the two sexes, which are grouped together in Tables II and III, were separated to con-

[3] The writers express their appreciation for suggestions made by Dr. O. A. Pope, U. S. D. A., Washington, D. C.

firm or disprove earlier conclusions, and the discussion is presented at another place in this article.

Tables IV and VI contain the results of the analysis of variance of some of the more important relations.

Table IV.—Computations Obtained in Calculating the Analysis of Variance of the Regional Marihuana Studies in 1939

Variance	D/F	Alkaline Beam Test Mean Squares	Alkaline Beam Test F Found	Alkaline Beam Test F For Odds 99:1	Acid Beam Test Mean Squares	Acid Beam Test F Found	Acid Beam Test F For Odds 99:1
Whole Plots							
Varieties	3	779.39	160.76	4.38	128.27	29.06	4.38
Locations	3	38.39	7.92	4.38	8.31	1.88	4.38
Var. x location	9	7.36	1.52	2.94	12.98	2.94	2.94
Block within location	12	5.07	1.04	2.72	4.99	1.13	2.72
Error (*a*)	36	4.84			4.41		
Split Plots							
Dates	2	40.42	19.17	4.92	580.01	217.90	4.92
Var. x dates	6	21.44	10.17	3.07	39.79	14.95	3.07
Location x dates	6	10.14	4.81	3.07	24.46	9.19	3.07
Var. x loc. x dates	18	6.14	2.91	2.28	9.65	3.62	2.28
Dates x block within loc.	24	2.51	1.19	2.07	4.68	1.76	2.07
Error (*b*)	72	2.11			2.66		
Total	191	17.64			14.49		

Table V.—Results Showing the Male and Female Plants Separated for Their Response to the Beam Tests

Variety	Sex	Mean	P. E.	Sex	Mean	P. E.	Difference Male	Difference Female
				Acid Tests				
Manchurian "A"	Male	1.50	± 0.07	Female	1.13	± 0.05	0.37	± 0.09
Manchurian "B"	Male	1.28	± 0.07	Female	1.06	± 0.06	0.22	± 0.09
African "C"	Male	2.59	± 0.09	Female	2.38	± 0.08	0.21	± 0.12
Kentucky "D"	Male	1.38	± 0.12	Female	1.19	± 0.10	0.19	± 0.16
				Alkaline Tests				
Manchurian "A"	Male	0.21		Female	0.00		0.21	
Manchurian "B"	Male	0.31		Female	0.19		0.11	
African "C"	Male	0.27		Female	0.10		0.17	
Kentucky "D"	Male	2.44	± 0.17	Female	2.19	± 0.14	0.25	± 0.22

DISCUSSION OF RESULTS

Influence of Region.—Of the four regions studied, three representing humid conditions yielded total influences somewhat similar with the alkaline test. The small differences which exist in regard to region between the "test response totals" for Virginia (128), Wisconsin (116) and Mississippi (111) are not statistically significant but each is significantly different from that obtained in Nebraska (203).

The differences between locations as observed by the acid test are not as great as those by the alkaline test. The greatest difference is between Nebraska (233) and Virginia (191) and the analysis shows that this difference barely gives odds of 19:1 of significance. It is, however, interesting to observe that Nebraska gives the highest value as it did also with the alkaline test.

As fertilizers have been shown to have little or no effect on the response of hemp to either the alkaline or acid Beam tests, the results obtained from the different regions may be considered as having been influenced more by climatic differences than by differences in soil. It is apparent that the soil at all locations could not be similar, and although differences hitherto unaccounted for may have been present in these experiments, it is reasonable to suppose that the greatest variations have been due to climate. Nebraska, the driest and highest in elevation, produced the strongest reactions and Mississippi, with little elevation and the most humid climate, produced the weakest Beam reactions. It would be well if these results could be substantiated by further investigations.

Influence of Varieties.—Varieties apparently produce greater differences and influences on the response to the acid or alkaline Beam tests than any other factor which has been studied. This will prove exceptionally fortunate from the breeding point of view if the chemical groups responsible for these tests may later be linked or correlated in some way with the physiologically active substance in Marihuana.

The most striking effect of varieties on the alkaline test results is the low intensities obtained with the varieties Manchurian "A" and "B" and African "C" in comparison with Kentucky "D." The average of the first three varieties is slightly less than 10 per cent of the Kentucky test. While the differences due to location are approximately twofold, they are with varieties tenfold. Mention has been made already that the first three varieties had been bred and selected especially for a low alkaline test, and that the Kentucky had not been selected for that factor.

The differences obtained with the alkaline test between any of the first three varieties in comparison with the Kentucky "D" are highly significant.

The difference between "A" or "C" and "B" are not sufficient to give odds of 19:1.

The acid "test totals" give an entirely different ranking of varieties in respect to their response. These results indicate the slight, if any, positive correlation between the two tests. The lowest ranking variety for the alkaline test, African "C," is by the acid test the highest. Its difference in comparison with any of the other three varieties or the mean of the other three is highly significant. The next greatest difference occurs between the two Manchurian selections, "A" and "B." This difference gives odds of more than 19:1 but not as much as 99:1 of being significant.

The importance of variety results presented above were substantiated for the alkaline test in an earlier investigation in which six varieties, five of which were different from the varieties used in this research, showed the most important difference was due to variety. It is to be noted, however, that varietal effect is markedly less in the acid test than in the alkaline test.

Influence of Dates of Sampling.—The alkaline test shows an increase in intensity with the age of the plant. The actual results are: first sampling, 132; second sampling, 193; and third sampling, 233. The differences between the first and third sampling and the difference between the first and second sampling yield odds of significance greater than 99:1 and the difference between the second and third harvest gives odds of at least 19:1.

The acid test yields an increase in intensity with the age of the plant, which is much stronger than the alkaline. The actual results are: first sampling, 118; second sampling, 233; and third sampling, 494. The differences between any two of these samplings are all highly significant, giving odds of more than 99:1.

These results are more striking and significant than those described in an earlier paper (1). In that investigation a significant decrease was obtained in the third harvest by the acid test after the second had increased over the first. It is believed that the response in general increases with age but occasionally factors may depress this reaction.

Influence of Sex.—Conclusions were reached in an earlier paper that if differences existed between male and female hemp plants with respect to variety and their response to the Beam tests they were small. In 1939 the sex was recorded only on plants harvested at the last sampling period. Varieties "A," "B" and "C" had such few plants that gave any response to the alkaline test that differences between males and females were not believed to be applicable to the common test of significance as measured by the probable errors. However, the probable errors of the means were determined for the variety "D" for the alkaline test and for the means of all the varieties for the acid test. These means and probable errors are given in Table V. The numbers of the populations involved in the means are small, *i. e.*, females 48 and males 32. It

Table VI.—Results Obtained by Petroleum Ether Extraction of Leaves of Varieties of Hemp Studied in the Regional Experiments, Together with the Variance and Covariance of the Resins and Acid Beam Test Numbers for Mississippi, Nebraska and Wisconsin

	% Resins Extracted by Petroleum Ether Varieties				Means by	
	Manchurian "A"	Manchurian "B"	African "C"	Kentucky "D"	Dates	Locations
Mississippi						
1st sampling Apr. 20	1.55	1.24	1.23	1.30	1.33	
2nd sampling May 18	1.95	1.79	2.80	2.23	2.19	
3rd sampling June 20	2.78	2.02	6.45	5.23	4.12	2.55
Nebraska						
1st sampling June 13	1.80	1.75	2.86	1.29	1.92	
2nd sampling July 10	3.81	2.95	4.16	3.62	3.63	
3rd sampling Aug. 9 & 24	4.21	4.62	6.69	5.23	5.19	3.58
Virginia						
1st sampling May 29						
2nd sampling July 3	3.35	2.91	4.37	4.29	3.73	
3rd sampling Aug. 15	4.86	4.27	6.54	5.07	5.18	
Wisconsin						
1st sampling June 28	2.28	2.23	2.77	2.21	2.37	
2nd sampling July 21	2.91	2.40	3.50	2.85	2.91	
3rd sampling Aug. 10 & 21	3.89	3.45	5.19	3.99	4.13	3.14
Variety means	3.03	2.69	4.23	3.39		

	Variance and Covariance Mississippi, Nebraska and Wisconsin Mean Squares			
	D/F	Resins	Acid No.	r
Varieties	3	3.5975[a]	522.1851[a]	0.959[a]
Locations	2	3.2357[a]	27.0833	0.987[a]
Varieties x locations	6	0.2085	54.8240	0.760[b]
Harvests	2	20.6094[a]	1603.8333[a]	0.999[a]
Harvests x varieties	6	1.0125[b]	180.8240[b]	0.735[b]
Harvests x locations	4	0.7222	114.6666	0.384
Harvests x locations x varieties (error)	12	0.2942	41.1296	0.733[a]
Total	35	2.0636	205.5143	0.870[a]

[a] Significant odds 99:1.
[b] Significant odds 19:1.

is not particularly surprising that small differences might occur but only in one case does the difference in results between males and females appear significant. This is the case of Manchurian "A" where the acid test produces odds of approximately 175:1 of significance, indicating the difference between males and females may be partly varietal. Although Table V shows results in which the males in every case were stronger than the females, previous results in 1938 were not as consistent in this respect. In fact, from six varieties tested only three had male plants stronger than the females. It is interesting to note that the male plants responded as strong if not stronger than the females. The general practice in the past of collecting the resins in Asia has been principally from the female plants.

It is difficult to conclude from these results that sex plays any important role in the response to either the alkaline or the acid test. Possibly small differences may exist and with a greater number of determinations future work may prove the small differences to be significant but from a practical point of view it seems doubtful if the differences which have been found here have any importance.

ALKALINE VS. ACID TEST

Mention has been made earlier in this article that there existed little or no positive correlation between these two tests. This is true if the sexes are grouped together or the females segregated from the males. Coefficients of correlation as determined for plants between the alkaline and acid tests were obtained on hemp plants grown in 1938. None of these proved large, *i. e.*, $r = 0.17 \pm 0.08$ for female plants of a China variety and 0.26 ± 0.08 for male plants of the same variety. Even smaller coefficients were obtained for plants of a Roumanian and Italian varieties.

QUANTITATIVE DIFFERENCES IN RESINS PRODUCED

Table VI shows the results obtained in extracting samples of leaves with petroleum ether. The results of the first sampling in Virginia are missing as the plants were too small at harvest to furnish material for petroleum ether extraction after the removal of material for the Beam tests. There is easily observed in Table VI the consistent increase in resins that takes place as the plants mature. Of the four varieties of hemp tested, African "C" gave approximately 57% more resins in extraction than Manchurian "B." Of the four regions studied, Nebraska gave approximately 40% more resins than Mississippi. The analysis of variance shows the marked influence that maturity, varieties and environment have on the production of the resins. The analysis of covariance presented in Table VI indicates the strong relationship between the resin results and the acid Beam test numbers which does not differ for different varieties, locations or harvests. No such marked relationship is indicated between the resin content and the alkaline Beam test response.

CONCLUSIONS

It is concluded that the climatic conditions under which *Cannabis sativa* is grown influence response to the acid and alkaline Beam tests but that this effect is small as compared with that of agronomic variety.

The quantity of resins produced by plants is influenced by plant maturity, variety and environment. The relationship between the resin content and acid Beam test numbers is strong and the same for different varieties, different locations and different harvests.

REFERENCES

(1) Wollner, *et. al.*, JOUR. A. PH. A., 27 (1938), 29–36.

(2) Matchett, *et. al.*, Scientific Edition, JOUR. A. PH. A., 29 (1940), 399–404.

(3) Unpublished observations, Bureau of Narcotics.

(4) Adams, *et al.*, *J. Am. Chem. Soc.*, 62 (1940), 196.

The Clone in Pharmacognosy

*By William J. Bonisteel**

The concept of the genus (1) was the basis for a symposium at the joint meeting of the Botanical Society of America and the American Society of Plant Taxonomists held at Indianapolis, December 29, 1937. Students of plant life have been working for centuries upon the problems of classification, and while the accomplishment has been great, much remains to be done and in some groups a satisfactory system may never be attained. The problem of species has always been a difficult one. With intergrading characters present, it is possible to have almost as many species as there are individuals. Species splitting can be carried to such an extreme that only a specialist in that field would be able to identify the plant in question.

The sub-species and its various forms are a grouping that is used extensively. In the current U. S. P., this system is followed in many cases. Under the drug, aconite, both sub-species and varieties are used in the defi-

* From the Botanical Laboratory of the Biology Department, Fordham University, New York, N. Y.

nition. In other cases hybrid is used under cinchona and rhubarb. The ecologist uses the word *ecad* to signify a form arising by adaptation to environment. Senna is an example of a plant which when placed under excellent conditions of environment (cultivation in this case) produces leaflets that are twice the size of the wild plants. The *variant* is a form arising from variation, the *mutant* from mutations. The *hybrid* is a form which arises from the union of two unlike gametes. Types of bacteria have been recognized for a long period of time. Races and strains are well established in the literature. It has been proposed that such races and strains should be numbered. Such a plan has many good points.

During the last few decades the plant breeder and geneticist have been developing new and improved forms of plant life. The cytologist has been furnishing valuable information which has led to the development of cyto-genetics, which is playing an important part in the elucidation of the many problems of taxonomy. The important fact that is being established by this group is the development of a system of pedigreed cultures that enable one to know definitely from which plant these new types were derived. It must not be overlooked that many of these forms, which the plant breeder is developing, may also occur in nature. Many plants fail to set seed and may only be propagated by asexual multiplication. While never clearly stated, it is the implied suggestion that many sub-species and varieties in the seed plants set seed or at least feebly so. On the other hand, many of these plants are sterile. Such sterile plants must have arisen from a seedling or seedlings which gave rise to the plants in question.

In a previous series of papers (2, 3), Sparks Aconite was investigated and found to be a sterile plant and such is to be designated as a *clone*. Cytological investigation showed that this plant was a triploid and three sets of eight chromosomes were found in the somatic tissues of the root tip. Two of the sets of eight chromosomes are alike and they are assumed to have arisen from a tetraploid aconite or from a diploid aconite in which one gamete was unreduced. The other set of eight chromosomes are assumed to be a gamete from a diploid plant. Seed production has never been observed in this clone. Neither has it set seed in numerous controlled close-pollinations or hybridizing pollinations. Controlled hybridizing experiments have led to the development of many aconites from diploid and tetraploid species. Many of the plants do not set seed due to failures in meiosis. A specific word should be used that clearly establishes the identity of such a plant.

The *clone* is the collective name for all the plants asexually reproduced by division, grafting, etc., from one (seedling) individual. Such a group of organisms are descended by mitosis from a common ancestor. The propagating of apples by grafting is merely a method of clonal propagation. The Ben Davis apple and Concord grape are recognized in the nursery trade as varieties when it would be more fitting to call them clones. The use of the term clone was suggested by Webber (4) in 1903. Stout (5) has discussed the question of the clone in considerable detail in the *Journal of the New York Botanical Garden*. Stout has shown that the "Balm-of-Gilead" poplar exists only as a female tree and that the plant has been propagated by cuttings. The evidence seems to indicate that such a tree arose from one individual seedling and has been propagated by repeated multiplications. Such a plant is clearly a clone. In the current National Formulary, the plant designated as Poplar Buds is given a species and generic rank. The plant was named *Populus candicans* by Alton in 1789. Both male and female flowers were described and it is evident that he was dealing with two distinct plants. The history of this poplar is so well treated in Stout's article that one should read the original for full details. Since this article was published in 1929, the Committee on Nomenclature should have been aware of this investigation and made the correction so clearly indicated in the monograph. Revision of both the U. S. P. and N. F. should be conducted so that they will be examples of the precision that the developments of modern research provide.

It is known that many of the hybrids of

rhubarb are sterile or at least set seed poorly. Both peppermint and spearmint are drug plants whose taxonomic positions are not clear. It is essential in dealing with economic plants that the constituents derived from them shall be uniform as far as is possible. In the genus Aconitum (3), it has been shown that many of the so-called species and varieties are subject to considerable variation in both their chemistry and cytology. The establishment of a clonal type of plant would assure uniform progeny and chemical constituents. In the life history of certain known clones no variation has been observed.

In "Standardized Plant Names" (6), the conception of the clone is not considered. Yet Sparks Aconite is listed as "*Aconitum Napellus, Spark's Variety.*" In the A. Ph. A. Monograph on Aconite (7), Sparks Aconite is listed as *Aconitum Napellus* var. *Sparkii.* Both of these citations are errors since the plant was named after a man by the name of Sparks and one cannot permit the cutting of a letter of a name according to the rules of nomenclature.

It is suggested that, in the monographs of drug plants, the word clone be used to designate those plants which are propagated vegetatively from one single seedling. That it is necessary to relate this plant, as far as it has been ascertained, to its nearest species is well understood.

REFERENCES

(1) Bartlett, Anderson, Greenman, Sherff and Camp., "The Concept of the Genus," *Bull. Torrey Bot. Club*, 67 (1940), 349.

(2) Bonisteel, Wm. J. "The Sterilty of Sparks Aconite," *Ibid.*, 67 (1940), 93.

(3) Bonisteel, Wm. J., "Polyploidy in Relation to Chemical Analysis," Scientific Edition, JOUR. A. PH. A., 19 (1940), 404.

(4) Webber, H. J., *Science*, II, 18 (1903), 501.

(5) Stout, A. B., "The Clone in Plant Life," *Jour. N. Y. Bot. Gard.*, 30 (1929), 25.

(6) "Standard Plant Names."

(7) "Aconite," A. Ph. A. Monograph No. 1, (1938).

"In almost everything, experience is more valuable than precept"—Quintillian

Drug Extraction. XXIII. The Extraction of Ipomea*

By William J. Husa† and Thomas J. Macek‡

As part of their study of the official resins Husa and Fehder (1) reported on the relative value of different menstrua in the extraction of ipomea. They found that alcohol was preferable to the alcohol-water (9:1) mixture recommended by the N. F. VI. In the present investigation a further study has been made on ipomea and a report is presented on the relative merits of different methods of extraction of the drug.

EXPERIMENTAL

Materials Used.—Ipomea, N. F., in moderately coarse (No. 40) powder obtained from two different sources was used. Drug *A* contained 20.51 per cent resin and 8.22 per cent moisture. Drug *B* contained 20.53 per cent resin and 8.54 per cent moisture. Alcohol was used as the menstruum.

Analytical Methods.—The resin content of ipomea was determined by a modified N. F. VI Assay Method (see Experiment 1). Resin determinations on liquids were made by the N. F. VI Method. Moisture was determined by the U. S. P. XI Method for drugs containing no constituents volatile at 100° C. To determine total extractive, an aliquot sample of liquid was evaporated to apparent dryness on a water bath, the residue treated with 2 cc. of absolute alcohol and again evaporated to dryness on a water bath; the residue was then heated in an oven at 100° C. until the weight was constant. The hygroscopic nature of the resin and residues necessitated the use of balance desiccators in the balance.

Experiment 1. Variation of Solvent in the N. F. VI Assay of Ipomea.—Since alcohol was found to be the better menstruum for the extraction of ipomea, the question arose as to whether the assay for resin content should be conducted according to the directions specified in the N. F. VI, wherein an alcohol-water (9:1) mixture is used as the extracting solvent, or whether alcohol should be employed instead. The following experiment was conducted to determine the efficiency of both alcohol and the alcohol-

---

* Presented before the Scientific Section, A. PH. A., Richmond meeting, 1940.

This paper is based on a thesis presented to the Graduate Council of the University of Florida by Thomas J. Macek, in partial fulfilment of the requirements for the degree of Master of Science in Pharmacy.

† Head Professor of Pharmacy, University of Florida.

‡ Holder of a University of Florida Graduate Assistantship, 1938–1940.

water mixture in extracting resin from ipomea following the assay procedure of the N. F. VI.

Four 10-Gm. samples of drug from source *A* were subjected to the N. F. VI assay for ipomea. Two of these were extracted with alcohol while the remaining two were treated with the alcohol-water menstruum. Four 10-Gm. samples of drug from source *B* were subjected to an identical treatment. Two 20-cc. aliquots were removed at 20° C. from each of the liquid percolates for determination of resin. Total extractive was likewise determined.

Table I expresses the results of the determinations in terms of per cent. Each figure represents the average of four separate determinations.

Table I.—Variation of Solvent in N. F. VI Assay

	Alcohol Used	
	Per Cent Resin	Per Cent T. E.[a]
Drug *A*	20.51	23.93
Drug *B*	20.53	24.29
	Alcohol-Water Used	
	Per Cent Resin	Per Cent T. E.[a]
Drug *A*	20.41	27.14
Drug *B*	20.35	27.76

[a] = Total Extractive.

Experiment 2. Maceration with an Excess of Alcohol.—An experiment was conducted to determine the time necessary for the establishment of equilibrium in the maceration of ipomea with an excess of alcohol, using the centrifuge method developed by Husa and Magid (2).

Into each tared, wide-mouth bottle of about 250-cc. capacity were placed 10 Gm. of drug and 90 Gm. of alcohol. The bottles were then placed in a cabinet at 23° to 26° C. for 15 minutes, during which time the bottles were shaken every 5 minutes. The bottles were then centrifuged at about 1000 r. p. m. for 10 minutes and allowed to stand 5 minutes. The clear supernatant liquid was decanted into tared, glass-stoppered bottles and weighed; the bottles containing the wet marc were also weighed. The macerate was adjusted to 20° C. before withdrawing samples for assay. The same technique was employed for 12-hour, 24-hour and 36-hour maceration periods except that the bottles were shaken at convenient irregular intervals. Drug from both sources was used in the experiment.

Calculations were made as follows: (Weight of drug taken for extraction) minus (weight of moisture in drug) minus (weight of total extractive in macerate) = (weight of dry marc). (Weight of wet marc) minus (weight of dry marc) = (weight of liquid imbibed by the marc). The loss of menstruum during the process was determined by comparing the total weights of the materials used with the combined weights of the macerate and wet marc.

The results in Table II are averages of duplicate assays of duplicate macerations.

Assuming that the liquid imbibed in the marc contained the same concentration of dissolved resin as the macerate, calculations were made to determine the distribution of the resin at different stages of the maceration. The results are given in Table III.

Table II.—Maceration with an Excess of Alcoho

Period of Maceration	Weight in Gm. of— Liquid Imbibed in Marc	Macerate	Total Extractive in Macerate	Resin in Macerate
Drug A				
15 min.	16.23	75.75	1.67	1.54
12 hr.	15.19	76.85	1.76	1.62
24 hr.	15.07	77.08	1.83	1.67
36 hr.	15.28	76.98	1.83	1.67
Drug B				
15 min.	12.25	79.57	1.64	1.46
12 hr.	12.45	79.85	1.81	1.60
24 hr.	12.48	79.82	1.84	1.66
36 hr.	12.78	79.56	1.83	1.68

Table III.—Distribution of Resin in Maceration with an Excess of Alcohol

Period of Maceration	Percentage of Resin In Liquid Imbibed in Marc	In Macerate	Left Undissolved in Marc
Drug A			
15 min.	16.38	74.84	8.78
12 hr.	15.99	78.98	5.03
24 hr.	16.28	81.23	2.49
36 hr.	16.58	81.08	2.34
Drug B			
15 min.	11.20	71.31	17.49
12 hr.	12.42	77.93	9.65
24 hr.	12.95	80.76	6.29
36 hr.	13.49	82.02	4.49

Experiment 3. Percolation.—Four percolators were used in this experiment. Three were cylindrical in shape, of Pyrex glass, 64 cm. in length and 4 cm. in internal diameter. The fourth percolator consisted of an ordinary glass, ribbed funnel of about 1-liter capacity. All were fitted with tubing and clamps for adjusting the rate of flow of percolate. The drug used throughout the experiment was from source *A* and 250 Gm. of it were used in each percolator.

Two of the cylindrical percolators were packed dry, the drug being introduced in small portions with slight agitation to promote even distribution, and when the entire portion had been introduced pressure was applied to the drug from the top. One of these percolators (Percolator 1) was packed very firmly from the top and the volume in the percolator occupied by the packed drug was 585 cc. The second percolator (Percolator 2) was packed less firmly and the volume occupied by the packed drug in this case was 625 cc. The third percolator (Percolator 3) and the funnel (Percolator *F*) were packed with drug previously moistened with 25 cc. of alcohol for each 100 Gm. of drug; pressure was applied to the top of the drug. The volume occupied by the drug in Percolator 3 was 600 cc. while in Percolator *F* it was 550 cc.

The menstruum was added to each of the percolators and when liquid reached the lower orifice the

flow was stopped for a maceration period of 24 hours. Percolation was then allowed to proceed so that percolate was delivered at the rate of about 10 drops per minute. A total of 750 cc. of percolate was collected from each 250 Gm. of drug in successive fractions of 125 cc., 125 cc., 250 cc. and 250 cc.

The time required for liquid to reach the lower orifice of each percolator was as follows: Percolator 1 (dry-packed), 261 minutes; Percolator 2 (dry-packed), 216 minutes; Percolator 3 (moist-packed), 140 minutes; Percolator *F* (moist-packed), 15 minutes.

The results of resin and total extractive determinations are expressed in Table IV.

Table IV.—Percolation of Ipomea

Fraction	In Percolate from Percolator 1	2	3	*F*
Resin Extracted in Gm.				
1 (125 cc.)	32.00	31.00	33.98	33.93
2 (125 cc.)	17.63	17.88	14.00	12.75
3 (250 cc.)	1.25	1.19	1.15	2.01
4 (250 cc.)	0.25	0.29	0.30	0.43
Total (750 cc.)	51.13	50.36	49.43	49.12
Percentage of Resin Extracted				
1 (125 cc.)	62.40	60.45	66.26	66.11
2 (125 cc.)	34.38	34.86	27.30	24.86
3 (250 cc.)	2.44	2.31	2.24	3.92
4 (250 cc.)	0.48	0.56	0.58	0.83
Total (750 cc.)	99.70	98.18	96.38	95.72
Total Extractive in Gm.				
1 (125 cc.)	34.13	34.75	37.65	37.88
2 (125 cc.)	20.25	20.13	15.70	14.30
3 (250 cc.)	2.09	1.71	1.64	3.15
4 (250 cc.)	0.69	0.69	0.71	0.89
Total (750 cc.)	57.16	57.28	55.70	56.22

Experiment 4. Forced Percolation through a Long Column of Drug.—Using Drug *A*, tests were conducted to determine the efficiency of extraction of ipomea by the method of forced percolation through a long column of drug. The apparatus previously described by Husa and Huyck (3) was employed. A long drug column (approximately 24 feet) was obtained by using six sections of tubes and six elbows all of flanged pipe made from Pyrex glass. When erected the apparatus stood vertically about four feet in height and each straight tube was attached to the next by means of the elbow. The dimensions of the tubes were as follows: length, 91 cm.; internal diameter, 2.5 cm.; thickness of wall, 0.5 cm.

The six tubes and elbows were packed with a total of 2000 Gm. of drug, moistened with a total of 500 cc. of alcohol. The moistened drug was packed into the apparatus with firm pressure using about 30 to 40 Gm. of drug for each packing portion. The volume occupied by the drug in the apparatus was approximately 4000 cc. The drug-filled tubes and elbows were joined together using interface joint gaskets made of sulfur-free gum rubber and held together by metal joint flanges. One end of the drug column was connected to a pressure tank containing 6000 cc. of the menstruum. The menstruum was forced through the drug with air pressure that was maintained at 5 to 6 lb. per sq. in. throughout the experiment. The percolate was collected in two fractions, each 1000 cc. in volume. Resin and total extractive determinations were made on these fractions. The entire experiment was repeated in exactly the same manner. The only variation noted between the two experiments was in the time required for the menstruum to pass through the column of drug and reach the orifice. In the first experiment this required 111 hours while in the second experiment 178 hours were necessary. The results are expressed in Table V.

Table V.—Forced Percolation through Long Column of Drug

A. Experimental Data

Experiment	Volume in Cc.	Gm. Resin	Gm. T. E.[a]
	Fraction I		
1	1000	292.5	316.5
2	1000	291.5	316.0
	Fraction II		
1	1000	115.2	129.2
2	1000	117.0	131.2

B. Percentage of Resin Extracted

Fraction	Experiment 1	2
I[b]	71.3	71.1
II[b]	28.1	28.5
Total[c]	99.4	99.6

[a] = Total Extractive.
[b] = 0.5 cc. per Gm. of drug.
[c] = 1 cc. per Gm. of drug.

DISCUSSION OF RESULTS

Variation of Solvent in the N. F. VI Assay of Ipomea.—The data in Table I indicate that assay for resin content of ipomea by the procedure outlined in the N. F. VI but using alcohol as the menstruum gives results slightly higher than when the N. F. VI menstruum (9 volumes alcohol–1 volume water) is employed. Within experimental error both menstrua may be considered equally efficient. In the present study alcohol was the menstruum used in the assays, since alcohol was the menstruum employed in the extraction operations. More total extractive is obtained with the N. F. VI menstruum than when alcohol alone is employed.

Maceration with an Excess of Alcohol.—The data in Tables II and III indicate that although the rate of extraction of resin from ipomea by macerating 10 Gm. of drug with 90 Gm. of alcohol may vary slightly with the source and sample of drug used, the

greater portion of the resin content can be dissolved from the drug in a 15-minute maceration period. The efficiency of extraction of resin increases slowly with increase in the duration of the maceration period so that at the end of 36 hours less than 5 per cent of the resin remains undissolved in the marc. In the case of ipomea, equilibrium is reached at the end of 24 hours of maceration.

In this respect ipomea varies somewhat with other drugs studied. Husa and Magid (2) showed that equilibrium in the extraction of alkaloids by maceration from belladonna root in Nos. 40, 60 and 80 powders was reached in 15 minutes. In the case of jalap, Husa and Fehder (4) found that equilibrium in the extraction of resin was reached within 15 minutes. On the other hand, Husa and Lee (5) attained equilibrium only after 36 hours of maceration of 10 Gm. of podophyllum with 90 Gm. of alcohol.

Extraction by Percolation.—The results in Table IV show that more than 95 per cent of the resin content of ipomea can be obtained in 750 cc. of percolate from 250 Gm. of drug. At least 90 per cent of the resin is found in that fraction of percolate numerically equal in cc. to the weight in Gm. of drug taken for extraction. Moistening of drug before packing is without notable advantage. Extraction of resin is more complete from drug packed tightly than from drug packed less tightly.

Forced Percolation through a Long Column of Drug.—Using the method of forced percolation through a long column of drug it was found that more than 99 per cent of the resin could be extracted in the first 1000 cc. of percolate from 1000 Gm. of drug. The air pressure employed for forcing the menstruum through the drug was maintained at 5 to 6 lb. per sq. in. throughout the extraction process. The moistened drug was packed in small successive portions with firm pressure.

Using the same apparatus, Husa and Huyck (3) obtained full-strength fluidextracts of belladonna root without resorting to the collection of various fractions of weak percolates. They employed a pressure of 25 to 35 lb. per sq. in. Husa and Lee (5) found that as much as 99 per cent of the resin of podophyllum could be extracted in the first 1000 cc. of percolate from 1000 Gm. of drug if the drug had been packed in small successive portions with moderate firmness; the extraction was less complete with looser packing and when breaks occurred in the drug column during percolation. They employed a maximum pressure of 30 lb. per sq. in.

SUMMARY

A comparative study was made of the extraction of ipomea by various methods. Alcohol was found as efficient as alcohol-water (9:1) in the N. F. VI assay for ipomea. In the process of maceration of ipomea with an excess of alcohol, the greater portion of resin was dissolved from the drug within 15 minutes. In percolation experiments at least 90 per cent of the total resin of the drug was obtained in 250 cc. of percolate from 250 Gm. of drug. More than 99 per cent of the resin was obtained in the first 1000 cc. of percolate from 1000 Gm. of drug using the method of forced percolation through a long column of drug.

REFERENCES

(1) Husa, W. J., and Fehder, P., JOUR. A. PH. A., 26 (1937), 319.

(2) Husa, W. J., and Magid, L., *Ibid.*, 23 (1934), 1187.

(3) Husa, W. J., and Huyck, C. L., *Ibid.*, 27 (1938), 290.

(4) Husa, W. J., and Fehder, P., *Ibid.*, 24 (1935), 615.

(5) Husa, W. J., and Lee, D. W., *Ibid.*, 28 (1939), 593.

Hydrogenated Oil as an Ointment Base

IV. Antiseptic Ointments*

By George W. Fiero and Ted A. Loomis†

Bacteriological methods have been used by many workers to determine the antiseptic value of ointments. Considerable work has been done on the antiseptic value of phenol ointment (1). Husa and Radin (2) reported that the activity of phenol ointment is de-

* Presented before the Section on Practical Pharmacy and Dispensing, A. PH. A., Richmond meeting, 1940.

† University of Buffalo, School of Pharmacy.

pendent upon the base. This being true, it would be apparent that other antiseptic ointments might vary in their activity with the ointment base employed. Hydrogenated oils, described in a previous paper (3) are valuable ointment bases because, although similar to lard physically, they do not develop rancidity as rapidly as lard.

EXPERIMENTAL

Bacteriological Technique.—The agar-cup method of the Food and Drug Administration was employed (4). Beef broth was prepared using 5 Gm. of beef extract, 5 Gm. of sodium chloride, 10 Gm. of peptone and water to make one liter. Nutrient agar consisted of 3% of agar dissolved in beef broth and adjusted to a p_H of 7. The test organism was a 24-hour broth culture of *Staphylococcus aureus*.

The sterile agar was melted, cooled to a temperature not exceeding 50° C. and inoculated with 1 cc. of culture to 100 cc. of nutrient agar. This was thoroughly mixed and 25 cc. of the mixture poured into Petri dishes and allowed to cool. Of course, all media and apparatus were sterilized by autoclaving. When solidified, a circular piece of agar 22 mm. in diameter was removed by means of a sterile modified cork-borer. This cavity was filled with the ointment being tested. The ointment was melted at as low a temperature as possible and poured into the cavity. The plates were incubated for 48 hours. After this time, they were examined for free zones around the samples. The absence of bacterial colonies in these zones indicated antiseptic activity, the width of the zone indicating relative efficiency.

Phenol Ointment.—Craw and Lee (1) found that U. S. P. phenol ointment (2%) was only slightly bacteriostatic and not a dependable antiseptic. On the other hand, they found that if the ointment was prepared with benzoinated lard, it had antiseptic properties. Since hydrogenated oils resemble lard, ointments were prepared using 2% of phenol dissolved in the molten hydrogenated oils. Data are shown in Table I. None of the ointments showed bacteriostatic effect.

Boric Acid Ointment.—Boric acid ointments were prepared using the official ointment base and in the same manner using ten per cent of the acid with hydrogenated oils. The data are shown in Table II. None showed appreciable bacteriostatic effect.

Table I.—Antiseptic Action of Phenol Ointments

Hydrogenated Oil	Softening Point	Iodine Value	Antiseptic Zone (Mm.)
Cottonseed 1	36.3	66.7	0
Cottonseed 2	37.2	62.0	0
Cottonseed 3	39.4	66.9	0
Cottonseed 4	29.5	..	Trace
Cottonseed 5	31.3	66.0	0
Cottonseed 6	35.7	62.0	0
Cottonseed 7	41.5	62.7	0
Cottonseed 8	29.8	70.0	0
Cottonseed 9	41.8	57.9	0
Cottonseed 10[a]	38.0	60.5	0
Cottonseed 11[a]	37.7	65.0	0
Cottonseed 12[a]	37.7	73.0	0
Cottonseed A	..	53.0	0
Cottonseed *B*	..	59.5	0
Cottonseed *C*	..	65.9	0
Cottonseed *D*	..	70.7	0
Cottonseed *E*	..	74.9	0
Soy Bean 1	36.8	70.3	Trace
Soy Bean 2	36.5	72.0	0
Soy Bean 3	35.9	72–76	0
Soy Bean 4	35.8	74.0	0
Soy Bean 5	40.8	83.0	0
Coconut 1	..	5.1	0
Coconut 2	32.4	4–12	0
Coconut 3	43.0	4–12	0
Coconut 4[b]	34.4	1.5–3.5	2
Palm Kernel	..	0.5–1	0
Peanut Oil	40.7	73.3	0
Sesame Oil	42.7	57.7	0
Sesame Oil *A*	..	61.5	0
Sesame Oil *B*	..	65.5	0
Sesame Oil *C*	..	67.8	0
Sesame Oil *D*	..	73.3	0
Sesame Oil *E*	..	78.9	0
Lard 1	38.0	51.0	0
Lard 2	38.1	58–61	0
Untreated Lard	..	54.2	0
U. S. P. Ointment	..	....	0

[a] A mixture, chiefly cottonseed oil.
[b] With lecithin.

Table II.—Antiseptic Action of Boric Acid Ointments

Hydrogenated Oil	Softening Point	Iodine Value	Antiseptic Zone (Mm.)
Cottonseed 1	36.3	66.7	0
Cottonseed 3	39.4	66–69	0
Cottonseed 4	29.5	....	Trace
Cottonseed 6	35.7	62.0	0
Cottonseed 11	37.7	65.0	0
Soy Bean 1	36.8	70.3	0
Soy Bean 3	35.9	72–76	0
Soy Bean 5	40.8	83.0	0
Coconut 1	..	5.1	0
Coconut 3[a]	34.4	1.5–3.5	Trace
Peanut	40.7	73.3	0
Sesame	42.7	73.3	0
Lard 1	38.0	51.0	0
Lard 2	38.1	58–61	1
Untreated Lard	..	54.2	Trace
U. S. P. Ointment	..	....	0

[a] With lecithin.

Ammoniated Mercury Ointment.—Ointments of ammoniated mercury were prepared with the official ointment base and by incorporating ten per cent of

Table III.—Antiseptic Value of Ammoniated Mercury Ointments

Hydrogenated Oil	Softening Point	Iodine Value	Antiseptic Zone (Mm.)
Cottonseed 4	29.5	..	12–13
Cottonseed 5	31.3	66.0	10–12
Cottonseed 9	41.8	57.9	11–12
Cottonseed 10	37.7	65.0	11–12
Soy Bean 1	36.8	70.3	12–13
Soy Bean 4	35.8	74.0	12–13
Soy Bean 5	40.8	83.0	11–12
Coconut 1	..	5.1	10–11
Peanut	40.7	73.3	12–13
Sesame	42.7	73.3	10–12
Lard 2	38.1	58–61	11–13
Untreated Lard	..	54.2	10
U. S. P. Ointment	..	..	8

ammoniated mercury with melted hydrogenated oils. The data are shown in Table III. All of these ointments possessed bacteriostatic properties; those prepared with hydrogenated oils being greater than the official ointment prepared with petrolatum, wool fat and wax.

SUMMARY

Ointments of phenol, boric acid and ammoniated mercury were prepared, substituting partially hydrogenated oils for the official ointment base. Bacteriological tests with these ointments using *Staphyllococcus aureus* as the test organism indicated that boric acid or phenol ointments prepared with the offiicial base or with hydrogenated oils possessed no bacteriostatic properties.

All ointments of ammoniated mercury were bacteriostatic. Those prepared with hydrogenated oils were superior to ointments prepared using the official base.

REFERENCES

(1) Craw, R. O., and Lee, C. O., *Pharm. Arch.*, 9 (1938), 1–16.

(2) Husa, W. J., and Radin, J. M., JOUR. A. PH. A., 21 (1933), 861–869.

(3) Fiero, George W., *Ibid.*, 29 (1940), 18–24.

(4) Ruehle, G. L. A., *U. S. Dept. Agric. Circ.* No. 198 (1931).

Poisons and Poisoners*

By W. H. Blome and Michael Zajac†

Poisonous substances have been known since ancient times but, as is true of many things, no one knows who first recognized materials possessing such properties nor when such discovery was made. Some surmise that shepherds and others observed that browsing animals regularly avoided certain plants although they ate others growing in juxtaposition to them. Undoubtedly primitive man had personal and unfortunate experiences as a result of contact with, or of consuming, certain plants.

The word poison harks back to the Latin *potio*, a drink, draft, potion—thereby relating it to pharmacy and medicine—with the connotation of a noxious or deadly drink. It has come to denote the active or deadly agent itself rather than a solution or other dilution of the harmful substance. And since poisons are the *raison d' etre* of the science of toxicology it may be mentioned in passing that the root "tox" has been traced to an ancient word meaning "bow" or "arrow," or, in a very broad sense, to a tool or implement used in killing. From this some have made the deduction that man's first knowledge of poisons concerned natural septic poisons, because many primitive peoples smeared their arrows, spears or other weapons with the blood or other parts of animals previously slain, and which therefore probably were infected. Experience taught them that wounds inflicted with missiles thus prepared proved more generally fatal than did those occasioned by clean weapons. With such observations as a basis, primitive man dipped his arrows in various juices, brews and concoctions of harmless and harmful agents and then noted the results of his experiments. Why some of the things he smeared on his arrow tips seemed to be more effective in killing animals or enemies, while others were ineffective, he did not know; the phenomenon therefore constituted a mystery which he ascribed to the supernatural and regarded with superstitious awe.

According to Lewin, only few favored men, priests and rulers, in ancient times, knew any poisons or their properties, and he reasons quite logically that conditions of early times were similar to those which exist at present among the primitive peoples of Africa, India, Guiana, West Indies, Brazil and other countries, where the fetish priests and chieftains have a knowledge of such substances. These men prepare the poisons and administer them to persons accused of crime, who thereupon are required to submit to the ordeal in which Providence ostensibly intervenes in favor of the innocent, but punishes the guilty by death. The actual outcome is dependent upon the wish of the one in authority. It is stated that in cases in which the ordeal (calabar) bean is given to one accused, the priest or chieftain selects a good, active bean, or one that he knows to be innocuous, to the end that the recipient shall die or live, as the "medicine man" elects. Lewin states incidentally that in the Fiji Islands there are professional poisoners (*Todesmänner*) who secretly administer cumulative cardiac poisons.

According to some versions, poisons received attention in Greek mythology. Hecate, who is confused with other goddesses, and whose parentage is not clear, is said to have had the power to confer upon mortals, also to withhold from them, certain favors and blessings, and to have had command over all the mystic powers of nature which included wild plants possessing medicinal as well as toxic properties. The treatment of certain diseases, also of snake bites, came within her sphere of activities. She is said to have discovered poisons.

Aconite, named after a small town in Heractea, according to a myth, was the foam from Cerberus, the 3- to 50-headed dog that guarded the entrance

---

* Presented before the Historical Section of the A. PH. A., Richmond meeting, 1940.

† Wayne University College of Pharmacy.

to the infernal regions. Medea, the wife of Jason, the hero of the mythical argonautic expedition, is related to have saturated the wedding dress of her rival, Glauce, with poison, thereby causing her death.

Poets also sang about poisons, as did Ovid (43 B.C.–17 A.D.) when he wrote that the arrows of Hercules were covered with the venoms of the Lerneian serpent.

Coming down to more recent times, Shakespeare, in "Romeo and Juliet," refers to poison and associates its illegal sale with a poverty-stricken apothecary, to the latter's discredit, in the following fashion:

Romeo: "I do remember an apothecary
And hereabout he dwells—which I late noted
In tattered weeds, with overwhelming brows,
Culling of simples; meagre were his looks,
Sharp misery had worn him to the bones;
.....Noting this penury, to myself I said—
And if a man did need a poison now,
Whose sale is present death in Mantua,
Here lives a caitiff wretch would sell it him."

Being a holiday the apothecary's shop is closed, so Romeo calls to him and offers him 40 ducats for "A dram of poison."

The apothecary answers:

"Such mortal drugs I have; but Mantua's law
Is death to any he that utters them."

But, upon being importuned to make the sale he says:

"My poverty but not my will consents,"

And later:

"Put this in any liquid thing you will
and drink it off; and if you have the strength
Of twenty men, it would despatch you straight."

Poisons are mentioned several times in the Scriptures but in all cases in an allegorical sense.

Competent toxicologists have stated that an unqualified definition of a poison that will withstand critical examination is almost impossible. Many states have laws which attempt to define such an agent, and base the definition largely upon the dose required to produce deleterious or deadly effect. However, such definitions are not entirely satisfactory since most substances in small amounts do little or no harm, but may kill if taken in larger quantities. Thus it may be pointed out that, while small amounts of common salt must be included in the day's food, a quantity of 60 grams or more may be harmful, and one four times as great may destroy the life of an adult. The question then as to what is or is not a poison depends upon the quantity taken into the body. Usually a poison is understood to be a substance which, administered in a small dose, is generally destructive of health or life. The general concept of a poison is necessarily relative. It must act through its own inherent chemical or other force, after absorption, not by any mechanical injury such as is induced by a lead bullet or by ground glass. Some authorities hold that there is no soluble substance that does not have poisonous effects if used in sufficiently large amounts. In this sense water may be a poison, and it has been demonstrated that water can be just that.[1]

The antiquity of poisons and of a knowledge of such substances is indicated by the writings of Menes, the first historical King of Egypt, about 2300 B. C.; Susruta, the author of one of the Indian Vedas and whose period in history is variously given as from 800 B. C. to the second century A. D.; Apollodorus, 3rd century B. C.; Heracleides of Tarentum, 250 B. C.; Nicander of Colophon, 200–130 B. C.; Mithridates, King of Pontus, 124–64 B. C.; Heras of Cappadocia, first century B. C.; Zopyrus of Alexandria, about 90 B. C.; Aelius Promotus, first half of the first century, A. D.; Dioscorides, 40–90 A. D.; Attalus III Philometer, first King of Pergamus, who reigned in 138; also the following for whom no dates are assigned: Nicomedes of Bithnia, Theologus Morus, Marinus and Sextus Empiricus.

Some of these men cultivated poisonous plants and immunized themselves, and their friends did likewise, by taking small daily doses; some also prepared "antidotes" which were renowned for centuries not alone as antidotes to poisons but as medicines for all known diseases—veritable panaceas. They made very practical experiments with these agents, not on animals as would be done nowadays, but on criminals and, in some cases, on members of their own families.

Among poisons known to these men may be mentioned opium, mushrooms and other noxious plants, snake venom, mineral substances, poisonous animals, especially serpents, and one from peach kernels.

The early classification of poisons naturally was unscientific. Susruta recognized stable (plants and metals) and mobile (animal) poisons, and listed 8 roots, 5 leaves, 12 fruits, 5 flowers, 7 barks, juices and extracts; 3 lacticiferous plants, 13 bulbs, the ash of *Phenasma* and, last, yellow arsenic. He classified animal poisons as the glance (look, or "evil eye," as of a wild animal), breath, teeth, claws, urine, excreta, semen, bones and bile. Almost all genera of animals were included, but serpents were the most numerous.

Other writers mentioned aconite, conium, hyoscyamus, veratrum, hydrocyanic acid, opium, mandragora, colchicum, certain fungi (mushrooms),

[1] *J. Am. Med. Assoc.*, June 10, 1922, page 1840.

cantharides, copper oxide and sulfate, mercury as cinnabar, lead oxides, antimony (form not stated), some varieties of honey—possibly produced from the nectar of poisonous plants—beetles, toads, salamanders, the sea-hare, elaterium, euphorbia and apocynum species, white and black veratrum, mezereum and menyanthes.

Legend has it that in India girls and women took increasing doses of poison (arsenic?) until they were so saturated with it that mere contact with them would cause one to lose all five senses and eventually die. They were known as "poison girls" (*puellae veneficae* or *puellae venenificae*). Susruta wrote about them as did Rhazes (10th century).

In early days poisons were used in the form of chopped, bruised or ground plants or plant parts, expressed juices or infusions or decoctions. The Egyptians are said to have known hydrocyanic acid which they obtained in dilute form by macerating peach kernels in water. A poisonous potion found its way from Egypt to Rome and, from the fact that those who swallowed it died almost immediately, it has been assumed to have depended upon hydrocyanic acid for its toxicity.

Capital punishment was inflicted by the sword or by hanging until a little before the beginning of the Christian era, when poison was introduced as an official means of execution. It was employed by the Greeks, and it may have served to popularize the use of toxic agents for the purpose of committing suicide. Those who objected to disfigurement by falling upon a sword or of effecting the same end by other mechanical means, or whose courage failed them at the critical moment, resorted to the poison cup which, in many cases, caused a painless death. In the East and in parts of Europe, suicide was not then regarded as it is by us now, but rather as something desirable, and in some places received official blessing. It is stated that the public officials of Marseilles and Massilia dispensed a potion containing cicuta (conium) to any and sundry who could convince the former that death was desired or desirable. Some of the "reasons" that were accepted as good and sufficient were very flimsy. Opium appears to have been employed in many such cases.

In India poisons were secretly administered for purposes of robbery and revenge, and in many cases they were given to some member of the family. Here, especially, secret assassinations by means of poisons became so numerous that to discourage the practice within the family circle, the Hindoo widow was required to sacrifice herself upon her spouse's funeral pyre.

In view of the more or less general apathetic attitude toward suicide and to the official execution of criminals by poisonings, it is not surprising that so-called criminal schools for the training of professional poisoners, men and women, developed their art and conducted secret assassinations on a business basis, quite in the open, from the 15th to the 17th century, especially in the Republic of Venice and in Italy. For a suitable consideration, these poisoners could be employed by anyone in high or lowly estate to remove some unwanted individual. So many public officials and others were removed during the Middle Ages by means of poisons smuggled into their food or drink, that court "tasters" were installed to taste of every dish before it was presented to the person in high office. The thought was that if the food contained any noxious substance, the effects would be noted by the taster and the more important personage saved. Tableware of "Electron" was used because it was held that such ware would tarnish upon contact with poison. Venetian glassware was used for the same purpose as it was claimed that it would shatter when filled with poisoned wine.

Charles IX of France had an abiding faith in the ability of a piece of unicorn's horn to reveal the presence of poison in wine, and he had a member of his household dip a piece of it into his wine before it was served to him. The idea was, although it had never been tested, that the horn would change color in the presence of poison. The fact is that there never was such an animal as the unicorn.

On this same fictitious basis he believed that the bezoar stone would serve as an effective antidote if for some reason the test with the unicorn's horn had been omitted. Finally, at the suggestion of Ambroise Pare, the king's surgeon, the efficacy of the stone was put to test. A man who had been condemned to death for theft was given a dose of arsenic, and a half hour later, a bezoar stone. The poor fellow died in agony, and the stone lost its reputation as an infallible antidote.

In "Claudius the God," Graves, the author of the volume, has Claudius say: "I was still extremely popular with the Guards and took so many precautions against assassination—a constant escort of soldiers, careful searchings for weapons, a taster against poisons at every meal—and my household was so faithful and alert, that a man would have had to be extremely lucky as well as ingenious to take my life and escape with his own."[2]

In Italy, secret assassinations were instigated by high government and church officials or by the actual government itself. The deliberations of a "Council of Ten" preserved written records giving the reasons for desiring the death of one or another, the names of the membership voting aye or nay on the question, as well as the fee to be paid. This "Council of Ten" on Dec. 15, 1513, received an offer from a Franciscan brother, John Ragubo, to do away with objectionable personages, for a pension of 1500 ducats annually for his first successful accomplishment; for each subsequent poisoning he was to receive an additional stipend as per an accompanying schedule. The Council, in January 1514, accepted the "patriotic offer," and decided that the Emperor Maximilian should be the first victim.

The schedule of "prices" was as follows: for the successful assassination of the Great Sultan 500; King of Spain 150; Duke of Milan 60; Marquis of

[2] Robert Graves, "Claudius the God and His Wife, Messalina," 1935. Random House, Inc., New York.

Mantua 50; and the Pope 100 ducats. In addition, the incidental expenses of travel to and from the scene of action, and bribery of "friends" and servants of the intended victim, were to be defrayed. The concluding statement was: "The farther the journey, the more prominent the men, the more it is necessary to reward the toil and hardship undertaken, and the heavier must be the payment." (Blyth.)

A notorious French poisoner was Marie Marguerite Marquise de Brinvilliers, born about 1630. Her paramour, Godin de Sainte-Croix, while in prison, may have learned about poisons and their preparation from a certain Exili or Eggidi, a noted Italian poisoner. The former conveyed the information so obtained to his mistress who then killed her father, two brothers and a sister in the hope of inheriting their property.

It appears that this woman maintained an elegant, demure and modest exterior which concealed a diabolical nature. She visited hospitals, comforted the sick and supplied them with sweetmeats and dainties which she had poisoned. This enabled her to watch the effects and thus to extend her practical knowledge of the action of these agents.

There was much speculation as to the identity of the poison this woman and her mentor, St. Croix, employed. An apothecary, Guy Simon, was engaged to determine its composition. At his time, chemical analysis was barely in its formative stage, and Simon was not able to accomplish the task he had undertaken as is clear from his statement that—"It is a terrible, diabolic, intangible poison."

Along with a number of her associates in her nefarious undertakings she was condemned to be burned. According to some historians she paid the supreme penalty while others, who were more nearly her contemporaries, hold that she evaded execution.

Exili or Eggidi, previously referred to, is said to have assisted a Madame Olympia, Queen of Rome under Innocent X, to poison many people (150) that she might inherit their property. He is believed to have instructed others in the fine art of poisoning.

A Sicilian woman named Tofana or Toffana had a solution known as *Acqua Toffana, Acquetta di Napoli*, also as *Acquetta di Perugia*, which she is said to have used frequently and effectively in Palermo and Naples. It was a solution of arsenic. She was finally arrested, tortured and probably executed in 1709.

It is stated that there were in Paris during the latter part of the 16th century, some 30,000 poisoners. Just how much of these stories of murders is fact and how much is fancy, may be another matter.

Catherine de Medici, born in Florence in 1519, a niece of Pope Clement VII, and the wife of Henry II, King of France, is accused of having poisoned a number of people, and to have been in large measure responsible for the St. Bartholomew's Day massacre.

During the reign of Claudius (10 B. C.–54 A. D.) a woman named Locusta was a prominent compounder and purveyor of poisons which she supplied with directions for use. It was she who prepared, at the instance of the Empress Agrippina, the second wife of Claudius, and the mother of Nero, the poison that was used to kill Claudius. It was concealed in mushrooms. Nero later availed himself of her services to get rid of his tutor Burrhus, also of Britannicus and his sisters, the son and the daughters of Claudius.

Back in early times and still more so when the science of toxicology began to make headway, attempts were made to find effective toxic substances that would produce effects or symptoms simulating those of common diseases, in order to avoid suspicion. The development of chemistry, and toxicology in particular, made crude poisonings difficult and hazardous because of the possibility of detection. More subtle means of doing away with an enemy or rival had to be found. Among many novel devices that are said to have been employed were poisoned gloves, boots, shirts, wigs and other articles of wearing apparel; snuff, perfumes, tableware, needles, and even books, and letters.

Among the prominent personages who are known or believed to have been murdered by means of poisons are Socrates, 5th century B. C., condemned by the populace and killed by means of hemlock; Titus, Roman Emperor, 40–81; Otto III, Roman Emperor and King of Germany, 980–1002; Pope Alexander VI, a member of the Borgia family, 1431–1503, died from poison he had intended for a cardinal; Pope Clement VII, 1480?–1534, passed away by inhaling the vapors of poisoned candles; Henry VII, killed by poison concealed in a communion wafer; Konrad, King of Naples, was murdered by means of a clyster administered by his brother Manfred. In addition to the last three, Lewin states that the Republic of Venice employed poisons to do away with Charles VIII, Ludwig XII, two emperors, three sultans, many dukes, cardinals and bishops, all for political reasons. The German Emperor Otto III was done away with by Stephania, the widow of the Roman governor, John Crescentius, through the agency of arsenic-poisoned gloves. Camilla, a girl to whom Claudius was betrothed, was poisoned by the latter's grandmother, Livia. LaWall adds the following names to the list: Pope Victor II, Christopher of Denmark, King John of Castile and Henry IV of France.

As indicated in the quotation from Shakespeare, laws were passed in many countries, and ordinances in many cities, intended to control the unbridled sale of poisons. However, in spite of these regulatory measures, there always were some who could supply the deadly materials for a consideration. Some statutes prescribed the death penalty for violations. In England, at one time, a strong feeling of resentment developed toward cowardly poisonings, largely because of a number of deaths that occurred in the household of the Bishop of Rochester, which were believed to have resulted from poisoning. An Act therefore was passed during the reign of Henry VIII which set up not alone the death penalty, but re-

quired that the guilty person be boiled in oil. Wriotherley's Chronicle of 1542 cites a case in which this penalty was imposed, in the following words:

> "This yeare, the 17th of March, was
> boyled in Smithfield ane Margaret
> Davie, a mayden, which had pouysoned
> 3 householdes that she dwelled in.
> One being her mistress, which dyed
> of the same, and one Darington
> and his wyfe, which she also dwelled
> with in Coleman Street, which dyed
> of the same, and also one Tinleys, which
> dyed also of the same."

Later on, although the death penalty was retained, the form of execution was changed to hanging. In 1598 two men who had been accused of concealing poison in Queen Elizabeth's saddle were hanged.

Undoubtedly many of the stories that have been told and retold and some of which are repeated here are fantastic especially in regard to the number of persons poisoned by one individual; nevertheless they probably represent but a small proportion of the numbers of people who have come to an untimely end by murderous poisons since many deaths never were suspected to be due to other than natural causes. We need not excite ourselves unduly over the official executions by poison since they may not have been less merciful than other forms of capital punishment then in vogue. Execution by burning, stoning, quartering; slow torture by the application of hot irons, pouring hot oil or molten lead into wounds; gouging out eyes, cutting off hands, arms or legs, or confinement in cages with wild animals certainly were not civilized means of ridding society of even desperate and malicious criminals. In recent years, and in the most enlightened and humane country in the world, execution of capital offenders has been accomplished by means of lethal gas. There has been decided opposition to this form of punishment, but there are always people who oppose the "Eye for an eye" dictum regardless of the heinousness of the crime that has been perpetrated.

In the World War mass murder was accomplished by the several combatants by the diffusion of irritant, corrosive, destructive poisonous gases. While the older peoples decimated their numbers on a small scale, modern nations in their "civilized" warfare carry on human destruction on an infinitely larger and more efficient scale.

BIBLIOGRAPHY

(1) *The Americana*.

(2) Authenrieth, Wm., "Die Auffindung d. Gifte u. Stark Wirkender Arzneistoffe," 3rd Edition, 1903. J. C. B. Mohr, Tübingen and Leipzig.

(3) Berendes, J., "Das Apothekenwesen," 1907. Ferd. Enke, Stuttgart.

(4) Berendes, J., "Die Pharmacie bei den Alten Culturvölkern," 1891. Tausch & Grosse, Halle a. S.

(5) Blyth, A. W. and M. W., "Poisons: Their Effects and Detection," 4th Edition, 1906. Charles Griffin Co., Ltd., London.

(6) Brockhaus, F. A., "Der Grosse Brockhaus," Leipzig.

(7) Brundage, A. H., "A Manual of Toxicology," 12th Edition, 1920. The Hy. Harrison Co., New York.

(8) "Compton's Pictured Encyclopedia." E. E. Compton & Co., Chicago.

(9) Frazer, J. G., "The Golden Bough," 1935. The Macmillan Co., New York.

(10) Gadamar, J., "Lehrbuch der Chemischen Toxikologie," 1909, Göttingen.

(11) Haggard, H. W., "The Lame, the Halt, and the Blind," 1932. Blue Ribbon Books, New York.

(12) "LaGrande Encyclopedia." H. Lamirault et Cie., Paris.

(13) LaWall, C. H., "Four Thousand Years of Pharmacy," 1927. J. B. Lippincott Co., Philadelphia.

(14) Leschke, Erich, "Chemical Technology," translated by Stewart, C. P., and Dorrer, O., 1934. Wm. Wood and Co., Baltimore.

(15) Lewin, Louis, "Lehrbuch der Toxikologie," 2nd Edition, 1897. Urban & Schwarzenberg, Wien and Leipzig.

(16) MacCulloch, "The Mythology of All Races," 1932. Marshall Jones and Co., Boston.

(17) McNally, Wm. D., "Toxicology," 1937. Industrial Medicine, Chicago.

(18) "'Medico,' Strange Ways of Poisoning," *The Australian J. of Pharm.* (Dec. 30, 1939), 1122.

(19) "Murray's English Dictionary," 1909. Clarendon Press, London.

(20) Murray, A. S., "Manual of Mythology," 1898. Henry Altemus, Philadelphia.

(21) Otto, F. J., "A Manual of the Detection of Poisons," translated by Wm. Elderhorst, 1862. Bailliere Bros., New York.

(22) Peterson-Haines-Webster, "Legal Medicine and Toxicology," 2nd Edition, 1923. W. B. Saunders Co., Philadelphia.

(23) Schmidt, Alfred, "Drogen u. Drogenhandel im Altertum," 1924. J. A. Barth, Leipzig.

(24) Seligmann, S., "Die Magischen Heil- u. Schutzmittel," 1927. Stecker & Schröder, Stuttgart.

(25) Thompson, C. J. S., "The Mystery and Art of the Apothecary," 1929. J. B. Lippincott Co., Philadelphia.

(26) Trumper, Max., "Memoranda of Toxicology," 1925. P. Blakiston's Son Co , Philadelphia.

(27) Underhill, F. P., "Toxicology," 1924. P. Blakiston's Son Co., Philadelphia.

(28) Wootton, A. C., "Chronicles of Pharmacy," 1910. Macmillan & Co., London.